LOAN DOCUMENT

PHOTOGRAPH THIS SHEET

DTIC ACCESSION NUMBER

LEVEL

INVENTORY 0

McClellan AFB SVE Off-Gas Characterization...

DOCUMENT IDENTIFICATION

1 Jul 96

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

DISTRIBUTION STATEMENT

HANDLE WITH CARE

ACCESSION FOR
NTIS GRA&I
DTIC TRAC
UNANNOUNCED
JUSTIFICATION

BY

DISTRIBUTION/

AVAILABILITY CODES

DISTRIBUTION	AVAILABILITY AND/OR SPECIAL	
A-1		

DISTRIBUTION STAMP

DATE ACCESSIONED

DATE RETURNED

DTIC QUALITY INSPECTED 4

20010116 025

DATE RECEIVED IN DTIC

REGISTERED OR CERTIFIED NUMBER

PHOTOGRAPH THIS SHEET AND RETURN TO DTIC-FDAC

DTIC FORM 70A JUN 90

DOCUMENT PROCESSING SHEET

PREVIOUS EDITIONS MAY BE USED UNTIL STOCK IS EXHAUSTED.

LOAN DOCUMENT

McCLELLAN AFB SVE OFF-GAS CHARACTERIZATION, LITERATURE REVIEW, AND TECHNOLOGY SELECTION

DRAFT REPORT

Prepared for

Armstrong Laboratory Environics Directorate
139 Barnes Drive, Suite 2
Tyndall AFB, FL 32403-5323

by

Battelle Columbus Operations
505 King Avenue
Columbus, OH 43201-2593

July 1, 1996

DEFENSE TECHNICAL INFORMATION CENTER
REQUEST FOR SCIENTIFIC AND TECHNICAL REPORTS

Title: AFCEE Collection

1. Report Availability *(Please check one box)*

☒ This report is available. *Complete sections 2a - 2f.*

☐ This report is not available. *Complete section 3.*

2a. Number of Copies Forwarded: 1 each

2b. Forwarding Date: July/2001

2c. Distribution Statement *(Please check ONE box)*

DoD Directive 5230.24, "Distribution Statements on Technical Documents," 18 Mar 87, contains seven distribution statements, as described briefly below. Technical documents MUST be assigned a distribution statement.

☒ DISTRIBUTION STATEMENT A: Approved for public release. Distribution is unlimited.

☐ DISTRIBUTION STATEMENT B: Distribution authorized to U.S. Government Agencies only.

☐ DISTRIBUTION STATEMENT C: Distribution authorized to U.S. Government Agencies and their contractors.

☐ DISTRIBUTION STATEMENT D: Distribution authorized to U.S. Department of Defense (DoD) and U.S DoD contractors only.

☐ DISTRIBUTION STATEMENT E: Distribution authorized to U.S. Department of Defense (DoD) components only.

☐ DISTRIBUTION STATEMENT F: Further dissemination only as directed by the controlling DoD office indicated below or by higher authority.

☐ DISTRIBUTION STATEMENT X: Distribution authorized to U.S. Government agencies and private individuals or enterprises eligible to obtain export-controlled technical data in accordance with DoD Directive 5230.25, Withholding of Unclassified Technical Data from Public Disclosure, 6 Nov 84.

2d. Reason For the Above Distribution Statement *(in accordance with DoD Directive 5230.24)*

2e. Controlling Office: HQ AFCEE

2f. Date of Distribution Statement Determination: 15 Nov 2000

3. This report is NOT forwarded for the following reasons. *(Please check appropriate box)*

☐ It was previously forwarded to DTIC on _____ *(date)* and the AD number is _____

☐ It will be published at a later date. Enter approximate date if known. _____

☐ In accordance with the provisions of DoD Directive 3200.12, the requested document is not supplied because: _____

Print or Type Name: Laura Peña

Signature: Laura Peña

Telephone: 210-536-1431

DTIC Control AD Number: M01-03-0599

CONTENTS

1.0 INTRODUCTION 1

2.0 SITE BACKGROUND 2
2.1 Site Description 3
2.2 Existing SVE System 3
2.3 Previously Investigated Technologies 5
2.3.1 Titanium Oxide Photocatalytic Destruction of Vapor-Phase Compounds 5
2.3.2 Flameless Thermal Oxidation of Vapor-Phase Compounds 6
2.3.3 Regenerable Adsorption of Vapor-Phase Compounds 6
2.3.4 Photolytic Destruction of Vapor-Phase Compounds 7
2.3.5 Elastomeric Polymer Filter Media for Vapor-Phase Compounds 7
2.3.6 Nonthermal Plasma Destruction of Vapor-Phase Compounds 8
2.3.7 Summary of Off-Gas Treatment Technologies 8

3.0 OBJECTIVES 9

4.0 SVE OFF-GAS CHARACTERIZATION 10
4.1 Mass Flowrates 10
4.2 Volumetric Flowrates 12
4.3 SVE Off-Gas Constituents 13

5.0 LITERATURE REVIEW 18
5.1 Degradation of SVE Off-Gas Contaminants: Microbiology 18
5.1.1 Cometabolism 20
5.1.2 Oxidation of Chlorinated Aliphatic Hydrocarbons by Methane-Oxidizing Bacteria 21
5.1.3 Oxidation of Chlorinated Aliphatic Hydrocarbons by Toluene- and Phenol-Oxidizing Bacteria 24
5.1.4 Oxidation of Chlorinated Aliphatic Hydrocarbons by Ammonia-Oxidizing Bacteria 27
5.1.5 Oxidation of Chlorinated Aliphatic Hydrocarbons by Propane-, Propene-, and Isopropylbenzene-Oxidizing Bacteria 28
5.1.6 Summary of Cometabolism 30
5.1.7 Direct CAC Degradation 35
5.1.8 Degradation of Additional Constituents: Dichlorobenzenes, BTEX, and Acetone 36
5.1.9 Anaerobic Reductive Dechlorination 36

6.0 VAPOR-PHASE BIOLOGICAL REACTOR TECHNOLOGY 37
6.1 Conventional Biofilters 38
6.2 Fixed-Film Trickling Filters (Biotrickling Filters) 40
6.3 Bioscrubbers 43
6.4 Suspended-Growth Reactors 44
6.5 Membrane Bioreactors 44

7.0 BIOGAS REACTOR TECHNOLOGY 45
7.1 Contaminant Mass Transfer Requirements 46

7.2 Requirements for Cosubstrate Feeding and CAC Degradation Rates 46
7.3 Alkalinity Requirements and pH Control 48
7.4 Flow Capacity 48
7.5 Capacity for Anaerobic Treatment Zones 49

8.0 TECHNOLOGY SELECTION 50
8.1 Cosubstrate Selection 50
8.2 System Configuration 52
8.3 Vapor Throughput 53
8.4 Liquid (and Biomass) Discharge 53
8.5 Upsets Due to Shock Loads 55
8.6 Reactor Plugging 55
8.7 System Optimization 56
8.8 Summary of Literature Review and Technology Recommendation 56

9.0 REFERENCES 58

APPENDIX A. A-1

FIGURES

Figure 1. Operable Units A through H at McClellan AFB 4
Figure 2. Mass Flowrates Versus Time 11
Figure 3. Biotrickling Filter Reactor System Configuration 54
Figure 4. Biotrickling Filter Reactor System Design A-1

TABLES

Table 1. Characteristics of Sve Off-Gas Treatment Technologies Demonstrated at McClellan AFB, California 9
Table 2. Off-Gas Contaminant Characterization 14
Table 3. Physical Constants for Contaminants Detected in the Off-Gas 15
Table 4. Contaminants Selected for the Laboratory Demonstration 17
Table 5. Primary Biological Degradation Processes for Halogenated and Nonhalogenated Gas-Phase Contaminants in the SVE Off-Gas Wastestream 19
Table 6. Advantages and Disadvantages of Different Cosubstrate-Oxidizing Bacteria for Cometabolic CAC Degradation 31
Table 7. Reported Maximum TCE Degradation Rates and TCE Concentrations Tested for Different Cosubstrate-Oxidizing Cultures 33
Table 8. Reported TCE Transformation Capacities of Different Cosubstrates 35

LIST OF ACRONYMS

AFB	Air Force Base
BTEX	benzene, toluene, ethylbenzene, and xylenes
CAAA	Clean Air Act Amendments
CAC	chlorinated aliphatic compound
Cal/EPA	California Environmental Protection Agency
CF	chloroform
COC	chlorinated organic compound
CT	carbon tetrachloride
DCA	dichloroethane
DCB	dichlorobenzene
DCE	dichloroethylene
DCM	dichloromethane
DCP	dichloropropylene
DO	dissolved oxygen
DoD	U.S. Department of Defense
DRE	destruction removal efficiency
U.S. EPA	U.S. Environmental Protection Agency
EPIC	Environmental Process Improvement Center
FBR	fluidized-bed reactor
FTO	flameless thermal oxidation
HAP	hazardous air pollutant
IPB	isopropylbenzene
ITP	Innovative Technology Program
MEBK	methyl isobutyl ketone
MEK	methyl ethyl ketone
MMO	methane monooxygenase
MW	molecular weight
pMMO	particulate MMO
sMMO	soluble MMO

NCOC	nonchlorinated organic compound
NETTS	National Environmental Technology Test Site
NPD	nonthermal plasma destruction
OUA-OUH	operable unit A - operable unit H
PAC	powdered activited carbon
PAH	polycyclic aromatic hydrocarbon
PASS	particulate acid scrubber system
PCE	tetrachloroethylene (perchloroethylene)
PD	photolytic destruction
RA	regenerable adsorption
SVE	soil vapor extraction
TCA	trichloroethane
TCE	trichloroethylene
TNMOC	total nonmethane organic compound
TOM	toluene *ortho*-monooxygenase
TOPD	titanium oxide photocatalytic destruction
TPH	total petroleum hydrocarbons
UV	ultraviolet
VC	vinyl chloide (chloroethylene)
VOC	volatile organic compound

1.0 INTRODUCTION

McClellan Air Force Base (AFB) in Sacramento, California, is part of the National Environmental Technology Test Site (NETTS) program. NETTS is a joint Department of Defense (DoD) and U.S. Environmental Protection Agency (U.S. EPA) program for the evaluation and testing of environmental technologies.

McClellan AFB uses soil vapor extraction (SVE) systems to remove contamination from soils. The SVE systems draw air through the pore spaces between soil particles, stripping away volatile organic compounds (VOCs) and generating a contaminated gas stream, referred to as the off-gas. The objective of the NETTS program, with respect to McClellan AFB, is to develop a treatment process to remove the VOCs from the off-gas before it is discharged into the environment. Several physical and chemical processes have been tested and demonstrated at McClellan AFB and are described in Section 2. The objective of this project is to evaluate and demonstrate a biological reactor system for treating the contaminated off-gas generated at McClellan AFB.

The treatment of the McClellan AFB SVE off-gases poses significant engineering and economic challenges. The large number of different VOCs in the off-gas, including chlorinated organic compounds (COCs), nonchlorinated aromatic and aliphatic compounds, and fluorinated Freon™ compounds, poses significant engineering challenges. First, the treatment process must be able to remove, and preferably destroy, a wide variety of different VOCs in the off-gas, including chlorinated aliphatic compounds (CACs), chlorinated aromatic compounds, nonchlorinated aliphatic and aromatic compounds, fluorinated Freon™ compounds, polycyclic aromatic hydrocarbons (PAHs), methyl ketones, and possibly long-chain hydrocarbons. Second, the treatment system must accommodate a wide range of concentrations of those compounds and the possibility of highly fluctuating concentrations, depending on the SVE system operation. Greater than 95% of the VOCs in the off-gas must be removed and/or destroyed. Third, the treatment system must be able to control pH or be resistant to corrosion due to the production of hydrochloric acid (HCl) and hydrofluoric acid (HF) via the release of chlorine and fluoride during the destruction of halogenated VOCs.

Several physical, chemical, and thermal technologies have been demonstrated at two of McClellan AFB's operating SVE systems with varying degrees of success. In spite of the fact that some of those technologies have achieved high destruction and removal efficiencies (DREs), their capital and operating costs tend to be very high, and a more cost-effective off-gas treatment technology is needed. Biological off-gas treatment has been suggested as an alternative off-gas treatment method for McClellan AFB.

Biological treatment systems have been used to treat a wide variety of chlorinated and nonchlorinated organic compounds, including benzene, toluene, ethylbenzene, and xylenes (BTEX); nonchlorinated solvents such as acetone and methanol; and CACs such as trichloroethene (TCE). However, the biological treatment of a gas stream with the very complex mixture of different VOCs present at McClellan AFB appears to be unique and poses one of the challenges of treating the off-gas stream biologically.

This report presents the results of the off-gas characterization study for the McClellan AFB SVE system, a review of pertinent literature for the biological treatment of the off-gas VOCs, and the selection of a treatment process/technology to be demonstrated in the laboratory and in the field at McClellan AFB. This report has been prepared by Battelle and Envirogen, Inc. Envirogen is subcontracted to Battelle for this project. Both Battelle and Envirogen have extensive experience working with biological treatment systems for the remediation of environmental pollutants, and also has in situ treatment experience. Battelle has extensive laboratory and field experience with in situ and ex situ treatment systems; Envirogen specializes in ex situ biological reactor processes, including gas-phase biological treatment. Both companies have experience treating chlorinated and nonchlorinated organic compounds.

Section 2 of this report presents the site background for McClellan AFB. The project objectives and specific objectives of this report are presented in Section 3. The off-gas characterization is presented in Section 4. The literature is reviewed in Sections 5 and 6, where Section 5 focuses on the microbiology of the degradation of the different off-gas contaminants and Section 6 focuses on bioreactor process technologies. In Section 7, the bioreactor technologies are analyzed and a process is selected and designed to meet the demands of the McClellan AFB SVE off-gas. Section 8 presents the references cited in the text and a bibliography of sources reviewed to prepare this document.

2.0 SITE BACKGROUND

McClellan AFB has been designated the Chlorinated Hydrocarbons Remedial Demonstration Site as part of the NETTS program. The McClellan AFB environmental program is committed to demonstrating and developing innovative technologies for the remediation of hazardous waste sites, as well as waste minimization, pollution prevention, and energy conservation. The overall objectives of the demonstrations are to generate full-scale operating, performance, and cost data for each technology. The demonstration projects provide the Air Force a unique opportunity to showcase innovative technologies that offer the potential to accelerate site remediation in a cost-effective manner. The demonstrations also

allow the Air Force to develop better working relationships with the Environmental Process Improvement Center (EPIC) and the broad-based Innovative Technology Program (ITP).

EPIC is a formal alliance among McClellan AFB, the U.S. EPA, and the California EPA (Cal/EPA). The center was officially established in October 1991 on the premise that the polluter and the regulator do not need to be at odds. By cooperating together, the Air Force and the regulatory agencies can promote effective environmental protection through innovative management, education, communication, and action.

The ITP is an ongoing program that is built upon cooperative partnerships dedicated to identifying and evaluating those technologies that may be feasible for widespread application. Each technology partner contributes to the overall process goal of generating defensible data on technology costs and performance that can be shared and disseminated.

2.1 Site Description

McClellan AFB is divided into eight "operable units," defined as Operable Units A through H (OUA through OUH) (Figure 1). The biological treatment process is tentatively proposed for OUD. The original SVE system at OUD was installed exclusively within Site S. In late fall 1994, an expansion was installed that included areas within Sites 2, 3, 4, and 5 (Sites 2 and 5 are shown in Figure 1). Site S has an area of approximately 9,200 ft^2 and lies in the northwest portion of the base. OUD contains at least 12 waste pits that were used for waste solvent and fuel disposal from the early 1940s through 1981. The waste disposal zone extends from 15 to 28 ft below ground surface and has been estimated to contain a minimum of 41,000 yd^3 of waste debris and contaminated soil. Remedial investigation data collected in 1985 and 1991 indicate that the pit at Site S was used mainly for disposal of spent solvents and fuels.

2.2 Existing SVE System

The full-scale SVE system installed at OUD includes 27 SVE wells, 39 piezometers, 5 vacuum pumps, and a catalytic oxidation system (cat-ox) followed by a particulate acid scrubber system (PASS). The SVE extraction wells are screened in three different strata known as the deep, intermediate, and pit zones. The SVE wells are connected to a manifold system through which the extracted vapors are conducted to air-water separators. The system was normally operated at a volumetric flowrate of 1,100 standard cubic feet per minute (scfm) until 1996 when the system was updated and expanded, and the nominal flowrate was lowered to 500 to 600 scfm based on the new system configuration and expansion.

The manifold carrying the off-gas from the new expansion wells is connected to the original deep-well manifold.

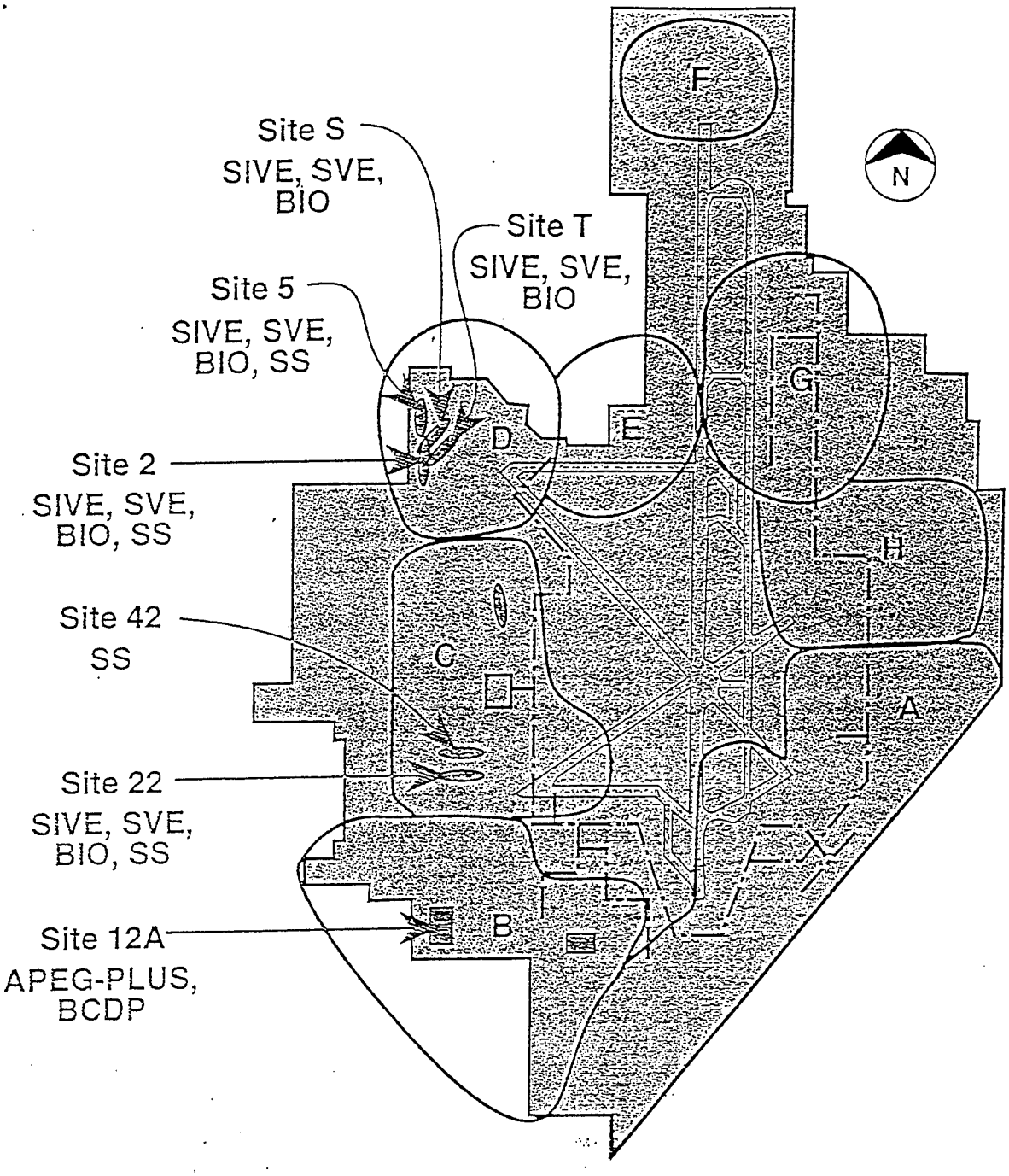

Figure 1. Operable Units A through H at McClellan AFB

The off-gas stream originating in the expansion wells at Sites 2, 3, 4, and 5 is higher in concentration than the stream from the older Site S wells. Typically, VOC concentrations in an SVE off-gas stream decrease over time as the soil residual concentrations decrease. Because the older wells at Site S had been extracting vapors for a longer time than the newly installed wells, it was reasonable that the off-gas stream from the expanded system had higher VOC concentrations than the older system.

2.3 Previously Investigated Technologies

As part of the innovative technology demonstration program, several off-gas treatment technologies have been studied at McClellan AFB. The objectives of the demonstrations are (1) to find cost-effective alternatives to the existing catalytic oxidation (cat-ox) and carbon adsorption off-gas treatment systems, (2) to determine the DRE of the different technologies, (3) to determine the reliability and ease of operation of those systems, and (4) to determine the ability of the systems to meet air quality regulations. Another point of interest in the demonstrations was to determine the concentration of nitrogen oxides (NO_X) in the effluent emissions. McClellan AFB is located in a nonattainment area for NO_X and is required to purchase "offset" credits for NO_X discharges.

The previously tested technologies include titanium oxide photocatalytic destruction of vapor-phase compounds, flameless thermal oxidation of vapor-phase compounds, regenerable adsorption of vapor-phase compounds, photolytic destruction of vapor-phase compounds, elastomeric polymer filter media for vapor-phase compounds, and nonthermal plasma destruction of vapor-phase compounds.

2.3.1 Titanium Oxide Photocatalytic Destruction of Vapor-Phase Compounds

The titanium oxide photocatalytic destruction (TOPD) process employed the interaction of ultraviolet (UV) light and titanium dioxide to produce highly reactive free radical ions. The free radicals react with VOCs to produce carbon dioxide (CO_2) and water. VOCs containing chlorine react to produce HCl, CO_2, and water. The reactor assembly was in the form of a tube lined with a mesh containing titanium dioxide and a UV lamp in the center. The off-gas from the SVE system was conducted through the tube reactor volume between the UV lamp and the titanium dioxide-containing mesh. The tube reactor assemblies were arranged in series.

The system was tested at a volumetric flowrate of 20 scfm. The effluent from the demonstration system was returned to the cat-ox/PASS treatment system to ensure adequate removal of contaminants prior to atmospheric discharge.

During the demonstration, the titanium dioxide reaction surface became fouled with a coating of long-chain (greater than 6 carbons) fuel hydrocarbons due to their high concentrations in the off-gas stream. Follow-up laboratory studies revealed that the high concentration of long-chain hydrocarbons made the TOPD technology unsuitable for the McClellan AFB off-gas stream.

2.3.2 Flameless Thermal Oxidation of Vapor-Phase Compounds

The flameless thermal oxidation (FTO) process utilized a ceramic matrix heated to 871°F to oxidize the contaminants in the off-gas stream to HCl, CO_2, and water. The temperature of the reaction chamber was regulated by microprocessor controls that were fed data by in-line thermal sensors that enabled the system to maintain stable operation with fluctuating flowrates and contaminant concentrations. The influent flowrate was maintained at 5 scfm.

The overall DRE for VOCs with the FTO system was 99.9% with production of less than 1 part per million (ppm) NO_X. The NO_X emission concentration from the FTO system was lower than from the cat-ox system. Cost analysis showed that the cost of operating the FTO system was $0.68/1,000 cubic feet of off-gas vapor compared to $0.99/1,000 cubic feet of off-gas vapor for a cat-ox system. Unit costs were based on monthly operating costs using an operating life of 5 years for both systems. In addition, the operational uptime for the FTO system was estimated at 95%, compared to 80% for cat-ox systems.

2.3.3 Regenerable Adsorption of Vapor-Phase Compounds

The regenerable adsorption (RA) process utilized a regenerable synthetic resin compound as an adsorbent to remove contaminants from the SVE off-gas stream at OUD. The demonstration system was operated at a flowrate of 180 scfm, and the effluent was returned to the existing off-gas treatment system. The demonstration system adsorbed contaminants onto the synthetic resin from the off-gas stream at ambient temperatures, and was regenerated by passing a hot purge gas over the resin bed. Two parallel beds were used to facilitate the regeneration of one bed while operating the other. The hot, contaminant-laden purge gas was then cooled to condense vapor-phase contaminants into the liquid phase.

The overall DRE for the system was calculated to be 95%. The removal of 11 pounds of contaminants from the off-gas stream resulted in the production of 2 gallons of condensed liquid

contaminant mixture. The condensate was approximately 50% water and 50% contaminants. The liquid was tested and found to be a hazardous waste by U.S. EPA and Cal/EPA standards. The demonstration system did not produce NO_X and HCl because contaminants were adsorbed instead of oxidized. The cost of operating the RA process was comparable to the cost of operating the existing off-gas treatment system.

2.3.4 Photolytic Destruction of Vapor-Phase Compounds

The photolytic destruction (PD) process, like the TOPD demonstration system, used UV light to oxidize VOCs into less complex and more reactive compounds (i.e., free radicals). Chlorinated compounds in the off-gas stream were oxidized into chlorinated free radicals, which were removed by reaction with a cement-like compound that lined the reaction chamber. The effluent from the system was primarily CO_2 and water.

The reaction chamber was arranged so that the off-gas stream flowed through a tortuous path within the reactor vessel under a plug flow regime. The off-gas stream was irradiated by UV lamps set at discrete intervals along the flow path. The barriers that forced the flow into the tortuous path were lined with a patented mixture of cement-like compounds that offered a reaction surface.

Extracted vapors from 8 of the OUD SVE system wells were conducted to the PD unit at a flowrate of 20 scfm. The effluent from the demonstration system was returned to the existing SVE off-gas cat-ox treatment system. The overall DRE for the demonstration system was 98% and was calculated using total nonmethane organic compound (TNMOC) concentrations. Chlorinated compounds were not included in the DRE calculations because their concentrations decreased steadily over time in the off-gas stream. The concentration of NO_X produced was 3 ppm and was less than the concentration produced by cat-ox systems. HCl production varied from 1.7 to 12.3 ppmv depending on the age of the liners in the reactor.

2.3.5 Elastomeric Polymer Filter Media for Vapor-Phase Compounds

The elastomeric polymer filter media process utilized a mixture of a molecular-bonding, cross-linked polymer, and activated carbon to adsorb contaminants from a SVE off-gas stream. Vapor from one SVE well was diverted to a canister containing the test filter media and then returned to the existing SVE off-gas cat-ox treatment system. Three tests were conducted in which the test filter media and flowrates were varied to determine the removal efficiency of the polymer.

Test results revealed that the polymer filter media had no advantage over activated carbon in the removal of the contaminants from the SVE off-gas stream. One test canister contained only polymer and no activated carbon, and did not remove any VOCs from the gas stream. Because the polymer-activated carbon mixture was more expensive than pure activated carbon, it was determined that no advantage would be gained by using the polymer mixture for treating the SVE off-gas.

2.3.6 Nonthermal Plasma Destruction of Vapor-Phase Compounds

The nonthermal plasma destruction (NPD) process system utilized a plasma-filled reactor to destroy the contaminants in the off-gas. Plasma is a physical state of matter in which molecules and atoms are in an ionic state. The plasma field was generated by inducing microbursts of high-voltage electrical energy across a dielectric (Pyrex) barrier. Chlorinated contaminant destruction by this method was expected to result in the production of hydrochloric acid.

Vapor from 8 SVE wells was conducted through the plasma destruction unit with a total volumetric flowrate of 10 scfm. The effluent from the demonstration system was returned to the existing SVE off-gas cat-ox treatment system prior to discharge.

Overall DRE was calculated to be 97% using TNMOC concentrations because of declining chlorinated VOC levels. The concentration of NO_X could not be determined due to unforeseen difficulties; however, previous experience by the operators on similar systems resulted in expected NO_X discharge concentrations around 2 ppm. The hydrochloric acid concentration measured in the demonstration system effluent was 150 ppm, indicating that an acid scrubber would be required to neutralize the effluent prior to discharge, similar to the existing system. An additional emission challenge of the NPD system was the high concentration of ozone in the effluent gas. Ozone is a primary contributor to smog in the lower atmosphere, and an ozone decomposer would probably be required as an additional effluent treatment step for the NPD off-gas stream.

2.3.7 Summary of Off-gas Treatment Technologies

Table 1 shows the DREs, NO_X production, HCl production, and cost of operating the six treatment technologies investigated; information that was unavailable is marked accordingly.

TABLE 1. CHARACTERISTICS OF SVE OFF-GAS TREATMENT TECHNOLOGIES DEMONSTRATED AT McCLELLAN AFB, CALIFORNIA

Technology	Site	Flowrate (cfm)	DRE (%)	NO_x (ppm)	HCl (ppm)	Cost, ($/1000 ft^3)	Comments
Titanium Dioxide Photolytic Destruction	OUC1	20	NA	NA	NA	NA	fouling by long-chain hydrocarbons
Flameless Thermal Oxidation	OUC1	5	99.9	<1	NA	0.68	
Regenerable Adsorption	OUD	180	95	ND	ND	~0.99	
Photolytic Destruction	OUD	20	98[a]	3	7	NA	
Elastomeric Polymer Filtration	OUC1	35	89	NA	NA	NA	DRE = 0 after 43 hours of operation
Nonthermal Plasma Destruction	OUD	10	97	NA	150	NA	ozone in effluent
Catalytic Oxidation/PASS	OUD OUC1	1,100	98		ND	0.99	existing treatment technology

NA = not available for this draft report
ND = not determined

3.0 OBJECTIVES

The overall objective of this study is to develop and demonstrate a biological treatment system capable of treating the off-gas stream from the McClellan AFB SVE system at OUD. The work is divided into two phases: Phase I includes the review and characterization of the existing SVE off-gas, review of current literature and available technologies, selection of one technology for further study, and preparation of a technology implementation plan for laboratory and field demonstrations of the selected technology. Phase II is divided into two parts, Phase IIA and Phase IIB, and will involve the execution of the technology implementation plan prepared in Phase I. Phase IIA will include a laboratory demonstration of the selected technology, and Phase IIB will include a field demonstration of the same technology. The objective of this report is to present the off-gas characterization results, to review the pertinent literature for biological treatment of the off-gas VOCs, and to select a process technology to be demonstrated in the laboratory.

4.0 SVE OFF-GAS CHARACTERIZATION

The SVE system at OUD has been operated since March 1993. On October 1994, the SVE system at OUD was expanded to include a larger area of contamination. The original SVE system withdrew vapor from wells installed exclusively within the area designated as Site S, while the new system was expanded to include wells and manifold piping at Sites 2, 3, 4, and 5, connected to the preexisting system deep-well manifold.

Raw data were obtained from McClellan AFB (Chapman, 1996) for the purpose of characterizing the individual VOC concentrations in the SVE off-gas stream. The data were collected between August 1995 and March 1996, from a sampling port in a manifold section that conducted off-gas from 8 newly installed monitoring wells in Sites 2, 4, and 5. The 8 wells were expected to produce relatively high off-gas contaminant concentrations (Chapman, 1989) and to represent a "worst-case scenario" for OUD. Off-gas VOC characteristics are summarized in Section 4.3 with respect to individual VOC concentrations and mass flowrates. The bioreactor technology selection is based on this data set. Because the system was operating under "worst-case" conditions, the data set should result in a conservative selection and design of the bioreactor for the removal and destruction of contaminants in the SVE off-gas.

4.1 Mass Flowrates

Figure 2 shows mass flowrates versus time; total petroleum hydrocarbons (TPH) are plotted against time from 16 March 1993 to 11 March 1996. The volumetric flowrates were relatively constant (approximately 1,100 scfm). At relatively constant volumetric flowrates, mass flowrates decreased from 80 to 90 lb/hr in April 1993 to 20 to 30 lb/hr in September 1994. In November 1994, the new extraction wells were brought online, and the mass flowrate increased rapidly to 70 to 90 lb/hr because new contaminated soils were exposed to the SVE gas flow stream. Between November 1994 and February 1996, the mass flowrates decreased, as expected, ending at a mass flowrate of approximately 5 to 10 lb/hr.

The decrease in extracted mass over time is typical of an SVE system, where organic compounds in the soil are increasingly difficult to volatilize over time because of decreased soil contaminant concentrations or because the most easily volatilized and mobile contaminants are stripped first,

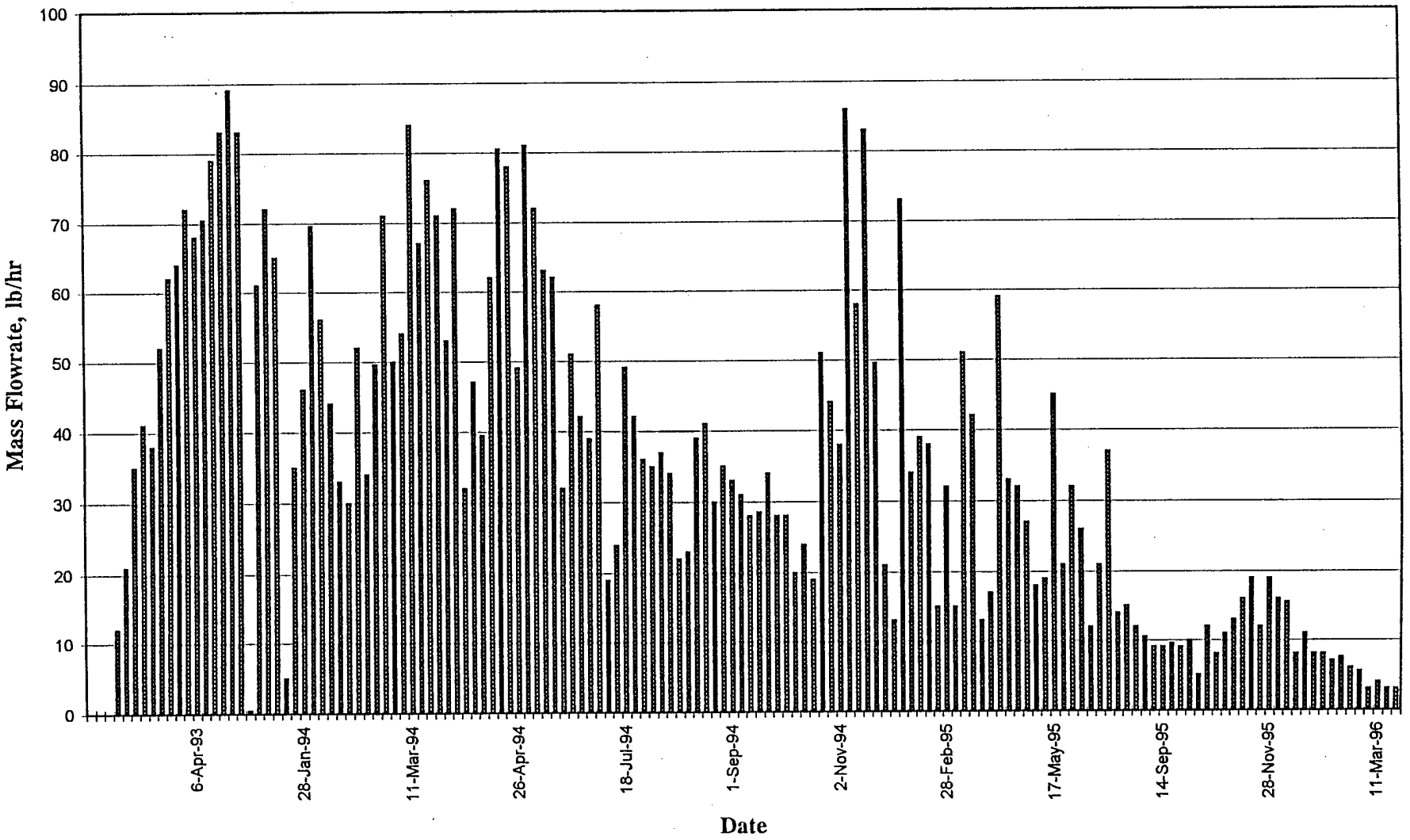

Figure 2. Mass Flowrates Versus Time

followed by those contaminants that are less mobile and less easily volatilized (Anderson, 1994). The fluctuations in TPH concentrations may be due to changes in flow vapor extraction rates in situ or, more likely, to changes in SVE operation from day to day. During periods when the system configuration and the arrangement of active extraction wells are kept constant, a decrease in mass flowrate typically is observed (Anderson, 1994). As different extraction wells are activated or inactivated, the concentrations in the off-gas stream change and the mass flowrate will show a sudden increase or decrease, respectively, followed, once again, by a steady decrease over time.

Most biological systems are sensitive to sudden increases or decreases in contaminant concentrations and/or mass flowrates. Such shocks can adversely affect biological processes and greatly reduce or eliminate the removal efficiency of the system. The SVE off-gas stream from OUD has the potential to produce shock loads, but those events are expected to occur with a high degree of predictability, depending upon the SVE system operation. Sudden increases in contaminant concentrations and mass loadings are expected to coincide with sudden changes in SVE operation, such as the initiation of new extraction wells to the off-gas stream. High concentrations also may occur in the event that an extraction well, or group of wells, that has been shut down for a period of time is suddenly brought online.

Shock loads are not expected to affect the biological system significantly for two reasons. First, because shock loads depend primarily on SVE system operation, they can be predicted in advance and can be managed to minimize the affects on the biological system. Second, the actual aqueous-phase CAC concentrations (« 5 mg/L for each VOC; refer to Section 4.3) are expected to be below their respective toxicity limits. Thus, increased gas-phase concentrations are not expected to result in toxic VOC concentrations in the aqueous phase.

4.2 Volumetric Flowrates

The old SVE system at Site S was operated at a volumetric flowrate of around 1,100 scfm, and was held relatively constant as wells were brought on- and off-line. With the introduction of the new expansion wells, the projected nominal volumetric flowrate for the current system is 500 to 600 scfm, with a maximum flowrate of 800 scfm (Chapman, 1996).

4.3 SVE Off-Gas Constituents

This section describes the constituents found in the off-gas stream produced by the expanded SVE system at OUD. The data set discussed here was collected under conditions of unusually high concentrations and, as such, are expected to result in a conservative design of the bioreactor treatment system. The off-gas consists of chlorinated aliphatic, chlorinated aromatic, and nonchlorinated organic compounds. TO-14 or EPA 8010/8020 analytical methods were used to quantify the off-gas contaminants; all three methods test for a wide range of organic compounds. Contaminant concentrations measured in the off-gas at OUD, between August 1995 and March 1996 are shown in Table 2; only those VOCs that were measured in the off-gas above the method detection limits are shown. The contaminants in the off-gas include chloroethenes, chloroethanes, chlorobenzenes, chloromethanes, trimethylbenzenes, Freon™ 113, BTEX compounds, acetone, and ketones.

Table 2 shows the minimum, maximum, and average concentrations measured in 9 to 29 samples. The number of samples for each contaminant depended on the method employed; method TO-14 measured for a wider range of contaminants than EPA-8010/8020. The number of sample hits for each compound also is shown. Some samples were consistently detected; for example, TCE was detected in 29 out of 29 samples. Other samples were detected less frequently; for example, chloromethane was detected in 1 out of 9 samples, and vinyl chloride (VC) was detected in 1 out of 28 samples. The minimum, maximum, and average off-gas concentrations are based on the actual sample hits rather than on the total number of samples. The standard deviation represents the deviation of the same set of samples.

The average SVE flowrate for all the samples was 1,045 scfm and was used to determine the mass flowrate in pounds of contaminant per hour (lb/hr). Aqueous-phase concentrations (mg/L) were determined using the average gas-phase concentrations and their respective Henry's law constants (atm-m^3/mole), assuming saturated conditions. Henry's law constants are shown in Table 3, along with other physical constants including molecular weight (MW), boiling point (^oC), solubility (g/L), and octanol-water partition coefficient (K_{ow}).

Chloroethenes measured in the off-gas included tetrachloroethene (PCE), TCE, 1,1-dichloroethene (1,1-DCE), *cis*-1,2-dichloroethene (*c*-DCE), and VC. TCE had the highest maximum (111 ppmv) and average concentrations (74 ppmv) of all the chloroethenes in the off-gas, followed by PCE (97 and 63 ppmv, respectively). The other chloroethene concentrations were below 10 ppmv.

TABLE 2. OFF-GAS CONTAMINANT CHARACTERIZATION

Compound	Off-gas Concentration Min. (ppmv)	Max. (ppmv)	Avg. (ppmv)	n/total	Stdev.	Mass Flowrate (lb/hr)	Conc. in H_2O (mg/L)
PCE	34.7	97.4	63.0	28/28	13.19	1.824	0.403
TCE	28.3	111.3	74.4	28/28	21.71	1.707	1.07
c-DCE	1.0	3.8	2.3	24/29	0.74	0.040	0.068
1,1-DCE	1.7	5.8	3.6	23/29	1.12	0.061	0.012
VC	0.2	0.2	0.2	1/28		0.002	0.001
1,1,1-TCA	53.9	241.4	152.9	29/29	46.64	3.564	4.15
1,1,2-TCA	0.8	0.8	0.8	1/9		0.019	0.092
1,1-DCA	1.6	5.2	3.6	28/29	0.89	0.062	0.065
1,2-DCB	1.2	120.6	35.5	27/29	34.57	0.911	2.82
1,3-DCB	1.4	5.2	2.8	5/9	1.54	0.073	0.235
1,4-DCB	1.6	21.5	6.6	8/9	6.26	0.168	0.651
Chlorobenzene	0.5	2.3	1.0	14/29	0.40	0.021	0.034
1,2,4-TMB	1.0	38.9	16.3	29/29	12.43	0.341	0.279
1,3,5-TMB	2.2	4.0	3.1	8/9	0.72	0.065	0.062
Methylene Chloride	1.9	7.8	5.4	28/28	1.73	0.080	0.225
Chloromethane	1.7	1.7	1.7	1/9		0.015	0.004
Freon™ 113	0.9	1.8	1.3	8/27	0.31	0.043	0.001
Benzene	0.1	5.4	2.8	2/29	3.77	0.038	0.040
Toluene	19.1	76.8	45.5	28/28	14.02	0.731	0.657
Ethylbenzene	1.2	8.3	3.6	27/28	1.81	0.067	0.046
Xylenes, Total	5.9	30.0	14.6	28/28	5.49	0.270	0.220
4-Ethyl Toluene	2.7	6.7	4.6	9/9	1.23	0.096	
Acetone	24.6	92.0	63.8	9/9	18.84	0.646	180
Methyl Ethyl Ketone	7.3	7.3	7.3	1/9		0.092	19.14
Methyl Isobutyl Ketone	6.5	21.1	17.5	9/9	4.73	0.306	42.08
Chlorinated Sum	134.7	669.8	374.4			**9.0**	**10.2**
Nonchlorinated Sum	67.3	247.6	159.5			**2.2**	**242.2**

Average Flowrate = 1044.8 scfm

TABLE 3. PHYSICAL CONSTANTS FOR CONTAMINANTS DETECTED IN THE OFF-GAS

Compound	M.W.	Boil. Pt (°C)	Solubility (g/L)	H (atm m^3/mol)	K_{ow}
PCE	165.83	121	0.15	2.59E-02	398
TCE	131.39	87	1.10	9.10E-03	194.98
c-DCE	96.94	60.3	3.50	3.37E-03	1.86
1,1-DCE	96.94	32	2.40	2.96E-02	134.9
VC	62.50	-13	2.80	1.09E-02	23.9
1,1,1-TCA	133.40	74	4.40	4.92E-03	309
1,1,2-TCA	133.40		4.50	1.17E-03	295
1,1-DCA	98.96	57.5	5.50	5.43E-03	61.7
1,2-DCB	147.00	180	0.15	1.85E-03	2398.8
1,3-DCB	147.00	173	0.11	1.78E-03	3981.1
1,4-DCB	147.00	174	0.08	1.48E-03	3311.3
Chlorobenzene	112.56	132	0.49	3.46E-03	691.80
Methylene Chloride	84.93	40	20.00	2.03E-03	20
Chloromethane	50.49	-24	NA	2.37E-02	8.13
Freon™ 113	187.40	NA	0.18	0.178	2000
1,2,4-TMB	120.19	170	NA	7.00E-03	NA
1,3,5-TMB	120.19	162 to 164	0.02	6.00E-03	NA
Benzene	78.11	80	1.77	5.40E-03	134.9
Toluene	92.04	111	0.53	6.37E-03	537
Ethylbenzene	106.17	136	0.16	8.39E-03	1412.5
Xylenes, Total	106.17	138 to 144	0.16-0.18	7.04E-03	318.3 to 584.9
4-Ethyl Toluene	120.20	162	NA	NA	NA
Acetone	58.00	56.2	imisible	2.06E-05	0.58
Methyl Ethyl Ketone	72.12	79.7	2.68	2.74E-05	1.88
Methyl Isobutyl Ketone	100.16	117.5	NA	4.16E-05	5.25

Chloroethanes detected in the off-gas included 1,1,1-trichloroethane (1,1,1-TCA), 1,1,2-TCA, and 1,1-dichloroethane (1,1-DCA). The 1,1,1-TCA appeared at the highest maximum (241 ppmv) average concentrations (152 ppmv) of all the CACs detected in the off-gas. The other chloroethane concentrations were below 10 ppmv.

Of the dichlorobenzenes (DCBs) detected in the off-gas (1,2-, 1,3-, and 1,4-DCB), 1,2-DCB had the highest maximum (121 ppmv) and average concentrations (35.5 ppmv). The average concentrations of the other DCBs were below 10 ppmv. Chlorobenzene also was detected at relatively low concentrations (1.0 ppmv average).

Chloromethane and methylene chloride both were detected below 10 ppmv, and the only fluorinated compound, Freon™ 113, was detected with an average concentration of 1.3 ppmv.

Of the nonchlorinated compounds, BTEX compounds totaled 66.5 ppmv, acetone concentrations averaged 63.8 ppmv, methyl ethyl ketone (MEK) averaged 7.3 ppmv, and methyl isobutyl ketone (MIBK) averaged 17.5 ppmv. Of the BTEX compounds, toluene had the highest average concentration of 45.5 ppmv.

The total sum of the average COC concentrations was 374 ppmv, resulting in a total aqueous-phase concentration of approximately 10 mg/L. The total sum of the average nonchlorinated organic compound (NCOC) concentrations was 160 ppmv, resulting in a total aqueous-phase concentration of approximately 242 mg/L. The very high aqueous-phase NCOC concentration is due primarily to the high projected acetone concentration of 180 mg/L due to acetone's high solubility and low Henry's constant.

Table 4 shows the COCs and NCOCs that will be used for the laboratory demonstration phase. The compounds shown in Table 4 were selected for two primary reasons. First, we selected VOCs that would represent the different groups of contaminants detected in the off-gas stream (i.e., chloroethenes, chloroethanes, chlorobenzenes, BTEX compounds, and acetone). The second criterion was to select the compounds that appeared at the highest concentrations in the off-gas. The compounds that were selected comprise approximately 85% of the total mass flowrate.

The compounds that will be tested in the laboratory phase include an aerobic-cometabolically degraded chloroethene (TCE), an anaerobically-degraded chloroethene (PCE), an aerobic-cometabolically degraded chloroethane (1,1,1-TCA), an aerobically degraded dichlorobenzene (1,2-DCB), an aerobically degraded BTEX compound (toluene), and acetone, which also is aerobically degraded.

TABLE 4. CONTAMINANTS SELECTED FOR THE LABORATORY DEMONSTRATION

Compound	Off-gas Conc. Max. (ppmv)	Off-gas Conc. Avg. (ppmv)	n/total	Mass Flow Rate (lb/hr)	Conc. in H_2O (mg/L)
PCE	97.4	63.0	28/28	1.824	0.403
TCE	111.3	74.4	28/28	1.707	1.07
1,1,1-TCA	241.4	152.9	29/29	3.564	4.15
1,2-DCB	120.6	35.5	27/29	0.911	2.82
Toluene	76.8	45.5	28/28	0.731	0.657
Acetone	92.0	63.8	9/9	0.646	180
Chlorinated Sum	570.70	325.80	0.00	8.01	8.44
Nonchlorinated Sum	168.8	109.3	35317	1.377	180.657

Trimethylbenzenes and ketones are not represented in Table 4. Those compounds also are expected degrade slowly. Freon™ 113 is another biologically recalcitrant compound that has been detected in the off-gas stream. Freon™ 113 concentrations in the off-gas averaged 1.3 ppmv, resulting in a projected aqueous-phase concentration of 0.001 mg/L.

One result of the high chlorinated and fluorinated compounds in the off-gas will be the production of HCl and HF compounds, which will produce acidic conditions in the wastestream. The complete mineralization of all the halogenated compounds in the gas stream at a flowrate of 1,000 scfm will result in the release of 71.5 kg Cl^- per day (2,000 mol/day) and 143 g F^- per day (7.5 mol/day) into the aqueous phase. This high release of HCl and HF compounds will require careful pH control and/or high alkalinity concentrations to buffer the pH of the system. Otherwise, the HCl and HF will result in acidic conditions that will be toxic to the bacteria, as well as corrosive to the system. In the laboratory-scale demonstration, the estimated HCl released into the reactors will be 1.8 to 3.6 g Cl^-/day (0.05 to 0.10 mol/day), assuming a gas flowrate of 0.05 to 0.1 scfm and assuming complete mineralization of all the organic chlorine. In the field demonstration, the estimated HCl released into the reactors will be 355 to 700 g Cl^-/day (10 to 20 mol/day), assuming a gas flowrate of 5 to 10 scfm and assuming complete mineralization of all the organic chlorine. The actual off-gas flowrates for the laboratory- and field-demonstration reactors will depend on the vapor throughput that can be achieved.

5.0 LITERATURE REVIEW

The literature review is divided into two sections: Section 5.1 is a review of the microbiology of off-gas contaminant degradation including chlorinated and nonchlorinated compounds; Section 5.2 is a review of gas-phase biological reactor treatment processes.

5.1 Degradation of SVE Off-gas Contaminants: Microbiology

This section focuses on the microbiology of contaminant degradation in the SVE off-gas. The results of the off-gas characterization show that the SVE off-gas consists of a very complex mixture of contaminants. It is important to understand the limitations as well as the potential advantages of the different biological processes for degrading the off-gas contaminants. The degradation characteristics of the off-gas contaminants are divided into the following groups: COCs degraded cometabolically, COCs and NCOCs degraded as primary growth substrates, and COCs degraded anaerobically via reductive dechlorination. Table 5 shows the compounds detected in the gas phase and the primary biological processes that contribute toward their degradation (i.e., aerobic degradation as a growth substrate, aerobic cometabolic degradation, and anaerobic dechlorination). Some of the organic compounds, such as BTEX, also can be degraded anaerobically, but they are degraded much more rapidly aerobically than anaerobically.

Most of the CACs are degraded fortuitously (i.e., cometabolically) by the action of nonspecific enzymes; those compounds are not used by bacteria as growth substrates for carbon and energy. Others can be used as growth substrates, and a third group falls into both categories. The chlorinated aliphatic compound TCE is a classic example of a compound that degrades only via cometabolism, while VC is an example of a compound that is cometabolically degraded or can serve as a source of carbon and energy for the bacteria. Finally, a fourth group of compounds exists that can only be degraded anaerobically, through a process known as reductive dechlorination. PCE is a classic example of a compound that is only degraded via anaerobic reductive dechlorination. PCE cannot be degraded aerobically as a growth substrate or cometabolically.

This section begins with a discussion of cometabolic degradation of chlorinated ethenes and ethanes, followed by a discussion of the growth-related aerobic degradation of other contaminants in the gas stream. The discussion of cometabolism is grouped by cosubstrate (i.e., methane, toluene and phenol, ammonia, propane, propene, and isopropylbenzene [IPB; cumene]). The last part of this section describes anaerobic dehalogenation for the halogenated compounds.

TABLE 5. PRIMARY BIOLOGICAL DEGRADATION PROCESSES FOR HALOGENATED AND NONHALOGENATED GAS-PHASE CONTAMINANTS IN THE SVE OFF-GAS WASTESTREAM

Off-gas Organic Compound	Primary Biological Removal Process		
	Aerobic Growth Substrate	Aerobic Cometabolism	Anaerobic Dechlorination
PCE			✓
TCE		✓	✓
c-DCE		✓	✓
1,1-DCE		✓	✓
VC	✓	✓	✓
1,1,1-TCA		✓	✓ a
1,1,2-TCA		✓	✓ a
1,1-DCA	✓	✓	✓ a
1,2-DCB	✓		✓
1,3-DCB	✓		✓
1,4-DCB	✓		✓
Chlorobenzene	✓		
1,2,4-TMB	✓		
1,3,5-TMB	✓		
Methylene Chloride	✓		✓ b
Chloromethane	✓		✓ b
Freon™ 113			✓
Benzene	✓		
Toluene	✓		
Ethylbenzene	✓		
Xylenes, Total	✓		
4-Ethyl Toluene	✓		
Acetone	✓		
Methyl Ethyl Ketone (MEK)	✓		
Methyl Isobutyl Ketone (MIBK)	✓		

[a] May be transformed abiotically under anaerobic conditions.

[b] May be degraded directly under anaerobic conditions.

5.1.1 Cometabolism

Bacteria that grow on hydrocarbons typically initiate oxidation by incorporating molecular oxygen into organic compounds by the action of enzymes known as oxygenases (Wackett and Householder, 1989). Two types of oxygenases, monooxygenases and dioxygenases, are involved in the biological oxidation of CACs. The mono- and dioxygenases typically are relatively nonspecific with respect to the types of organic compounds that they will attack. The fortuitous oxidation of CACs by bacteria is termed cometabolism.

The oxygenase enzymes do not specifically cleave carbon-halogen bonds, but produce unstable epoxide intermediates that release halides via further chemical or biological decomposition. It is generally accepted that the microorganisms implicated in cometabolism of CACs do not gain energy or carbon for cell growth from the cometabolic oxidation of CACs. In fact, cometabolism often results in the depletion of stored energy reserves in the cell (Alvarez-Cohen and McCarty, 1991). This implies that an organic cosubstrate other than the CACs is required for biological growth and for the production of the necessary oxygenase enzymes that are used to degrade the CACs. A variety of growth substrates have been used for cometabolic CAC degradation, including methane, aromatic compounds (toluene and phenol), propane, propene, and IPB (cumene). The advantages and disadvantages of each of these cosubstrates will be discussed.

Important issues related to the engineering application of cometabolic CAC degradation include CAC toxicity, competitive inhibition, and intermediate toxicity due to CAC degradation by-products. In particular, many CACs have solvent properties that can adversely affect cell membranes, enzymes, and proteins (Bielefeldt et al., 1995). Because CACs are degraded by the same nonspecific enzymes responsible for the degradation of the growth substrate, competition between the CAC and the growth substrate is known to occur. Competition can reduce the degradation rates of both the growth substrate and the CAC. In addition, multiple CACs can compete with each other (Strand et al., 1990), resulting in reduced CAC degradation rates.

Intermediate toxicity occurs when a by-product of CAC degradation exerts toxic effects on the cells (Alvarez-Cohen and McCarty, 1991; Bielefeldt et al., 1995). For example, methanotrophs convert TCE to TCE-epoxide (Fox et al., 1990), which can react with and damage intracellular cell protein and DNA (Wackett and Householder, 1989). As the amount of TCE transformation increases, the amount of TCE-epoxide increases and the viability of the cell decreases until it is lost entirely. Intermediate toxicity poses a significant treatment challenge with respect to cometabolic degradation of CACs. Direct

TCE toxicity also is a concern; like many organic solvents, TCE is hydrophobic and can express toxic or inhibitory effects on cells by partitioning onto cellular components, such as lipids and polysaccharides. However, the toxic effects of TCE appear to be much less significant than the effects of intermediate toxicity during TCE degradation.

The following discussion of cometabolic degradation of CACs using different cosubstrates focuses on (1) the ability of the substrate-specific bacteria to degrade different types of CACs (i.e., mono-, di-, and tri- chloroethenes and chloroethanes), (2) competition between the organic cosubstrate and CACs, and (3) intermediate toxicity during CAC biotransformation.

5.1.2 Oxidation of Chlorinated Aliphatic Hydrocarbons by Methane-oxidizing Bacteria

Wilson and Wilson (1985) were the first to demonstrate the biological cometabolism of TCE, using ^{14}C-labeled TCE; TCE was transformed to CO_2 in a soil column that had been exposed to natural gas with 74% methane. The authors concluded that methanotrophic organisms (the methane-utilizing bacteria) were responsible for the transformation of TCE, and TCE degradation has since been confirmed in numerous other studies using methane-oxidizing bacteria.

Methanogenic bacteria grow aerobically, using methane as a carbon and energy source. The oxygenase enzyme methane monooxygenase (MMO) catalyzes the first step in the biochemical pathway of methane oxidation. MMO requires both oxygen and a high-energy reducing substrate (NADH) to catalyze the oxidation reactions. Formate can be used as an alternative energy source to support intracellular NADH production, but it does not support the production of MMO enzymes, which require methane as the primary substrate (McFarland et al., 1992). In some methanotrophs, MMO is nonspecific and can catalyze the cometabolism of a number of chlorinated aliphatic chemicals, including chloroethenes, chloroethanes, and chloromethanes.

Oldenhuis et al. (1989) showed that two different MMO enzymes are responsible for methane oxidation: Type 1 methanotrophs produce a soluble form of MMO (sMMO), and Type 2 methanotrophs produce a particulate form of MMO (pMMO). TCE was degraded only by cells that expressed sMMO, which demonstrated a much broader substrate range than pMMO. The sMMO was expressed only under low copper concentrations (< 0.25 μM copper [Tsien et al., 1989]); and, when copper was added to the medium, only pMMO was expressed, and no TCE degradation was observed. Oldenhuis et al. (1989) also showed that sMMO catalyzed the degradation of a wide variety of other CACs, including chloroform (CF) and dichloromethane (DCM); 1,1-DCA, 1,2-DCA, and 1,1,1-TCA; 1,1-DCE, *c*-DCE, *t*-DCE, and TCE; 1,2-dichloropropane; and 1,3-dichloropropylene (*t*-1,3-DCP).

Methanotrophic bacteria have been shown to degrade TCE, DCE, and VC at relatively high degradation rates to nondetectable concentrations by a variety of pure and mixed methanotrophic cultures (Oldenhuis, et al., 1989; Alvarez-Cohen and McCarty, 1991; Tsien et al., 1989; Fogel et al., 1986). The ability to degrade a wide variety of CACs and the ability to rapidly degrade TCE made methanotrophs the subject of further studies with the potential to use them to degrade CACs in the environment.

In spite of the success reported using methanotrophs to cometabolically degrade a wide variety of CACs, including TCE, the application of methanotrophs for the removal of CACs from the environment is restricted by three main factors (Oldenhuis et al., 1989). First, not all methanotrophs can degrade TCE; some organisms, for example, produce exclusively pMMO and as such are unable to degrade TCE. Second, methane inhibits TCE degradation; during methane-supported growth, methane inhibition could limit TCE degradation. Third, TCE degradation intermediates are toxic to methanotrophs.

In a mixed methanotrophic culture, only a fraction of the overall biomass will contribute directly to the degradation of TCE and other CACs, while the entire population will be dependent on methane as a growth substrate. Due to the nature of cometabolism, TCE degradation provides no selective advantage to methanotrophs, and it is improbable that selective enrichment of TCE-degrading methanotrophs under field conditions can be achieved (Oldenhuis et al., 1989). The design and operation of a methanotrophic TCE-degrading culture must be one that enhances sMMO production and stimulates the reducing power (i.e., intracellular NADH production) of the culture, but the presence of other methanotrophs that will compete for methane cannot be avoided. Methanotrophs exhibit first-order TCE degradation rates. First-order degradation rates imply that long treatment periods or high cell concentrations are essential if low final concentrations are required. The fact that only a fraction of the overall biomass will contribute to TCE (or CAC) degradation implies that a large fraction of the biomass will remain unused with respect to CAC degradation.

Because MMO is responsible for both methane oxidation and TCE epoxidation, methane and TCE are considered to be competitive substrates (Alvarez-Cohen and McCarty, 1991). In the presence of methane, TCE degradation rates are reduced. In column studies using soil microcosms, Lanzarone and McCarty (1990) showed that little or no TCE degradation occurred when the feed methane concentration was 4.5 mg/L, but TCE degradation did occur with a feed concentration of 1.5 mg/L, suggesting competition between methane and TCE for MMO activity. Balancing the drawbacks of enzyme competition and the requirement of methane for growth and maintenance represents a significant engineering challenge. The kinetics of methane degradation and TCE degradation are such that the MMO enzyme

has a much higher affinity for methane than for TCE, so that relatively low methane concentrations can inhibit TCE degradation rates. In the presence of low TCE degradation rates, a large active biomass must be supported to maintain significant TCE biodegradation (Speitel and Leonard, 1992). However, the production of a large biomass culture also requires high methane concentrations, resulting in a viscous cycle of increasing methane concentrations and decreasing TCE degradation kinetics.

The problem of methane and TCE competition has been overcome in the laboratory by using resting cells; cells are grown first on methane and subsequently are fed TCE after the methane is depleted. Once the methanotrophs exhaust their ability to degrade TCE via the exhaustion of MMO enzymes and intracellular NADH, they are subjected to another feed cycle using only methane. Simulating a batch feed cycle in the field is difficult in part because methanotrophic bacteria do not always recover from intermediate TCE toxicity after repeated batch feed cycles (Alvarez-Cohen and McCarty, 1991).

The oxidation of TCE also results in a TCE-epoxide intermediate, which is subsequently degraded to CO_2 and HCl (Little et al., 1988). TCE-epoxide intermediates have been implicated in toxic inhibition of TCE-degrading methanotrophs (Alvarez-Cohen and McCarty, 1991; Henry and Grbić-Galić, 1991; Oldenhuis et al., 1989). The toxicity effects can be permanent, and the methanotrophic bacteria often have difficulty recovering from toxicity caused by TCE-epoxide formation. Even at low concentrations, TCE transformation can result in significant decay of the active biomass, which would be expected to increase with the increasing amounts of transformed TCE (Henry and Grbić-Galić, 1991). In addition, different methanotrophs may be affected by TCE oxidation toxicity to different degrees.

Because of competition and product toxicity, methane degradation and cell growth may not coincide with TCE degradation (Semprini et al., 1990). The development of a two-step system could be required, in which the first step involves growth of methanotrophs under controlled conditions via methane (natural gas) degradation, and the second step involves TCE degradation supported by an alternative energy source for the methanotrophs (i.e., formate, McFarland et al., 1992). Another possibility could be to cycle back and forth between methane growth and TCE degradation, using multiple-sequence batch reactors in parallel with their degradation and growth modes out of phase.

A 2-stage system was investigated by McFarland et al. (1992), where methanotrophs were grown in a separate enrichment reactor and fed into a TCE-degrading reactor. Over 95% TCE degradation (from 29.2 to 1.4 mg/L TCE) was observed, with a volatile solids concentration of 1.3 g/L in the TCE degrading reactor. The mixed methanotrophic culture was ineffective in biodegrading TCE unless it was supplied with formate as an energy source. In the absence of formate as an energy source for NADH, the

removal of TCE occurred mainly through sorption. Unfortunately, the experiment was not conducted over long time periods to demonstrate the long-term ability of methanotrophic cultures to sustain TCE degradation.

Because of the problems described above (first-order degradation rates, competition between methane and TCE, intermediate toxicity), other cosubstrates have been investigated, and their performance has been compared to that of methane.

5.1.3 Oxidation of Chlorinated Aliphatic Hydrocarbons by Toluene- and Phenol-Oxidizing Bacteria

Cometabolic TCE degradation is catalyzed by toluene-oxidizing cultures (Hecht et al., 1995; Mu and Scow, 1994; Wackett and Gibson, 1988) and phenol-oxidizing cultures (Hopkins et al. 1993a; Hopkins et al. 1993b; Folsom and Chapman, 1991; Folsom et al. 1990). Similar to the methane-oxidizing bacteria, which generate the nonspecific enzyme MMO for the initial oxidation step toward methane degradation, the toluene- and phenol-degrading bacteria produce oxygenase enzymes, which are used for the initial oxidation of toluene or phenol degradation, respectively. Those dioxygenase enzymes, like MMO, are relatively nonspecific and have been shown to degrade TCE as well as all three DCEs (Bielefeldt, 1994; Shields and Reagin, 1992; Wackett and Gibson, 1988). However, unlike MMO, they do not appear to be able to degrade chloroethanes (Hopkins et al., 1993; Shields and Reagin, 1992) or VC and ethylene (Wackett and Gibson, 1988).

Nelson et al. (1988) showed that two strains of *Pseudomonas putida*, Strain PpF1 and Strain B5, utilized a toluene dioxygenase enzyme as the initial step in TCE degradation. Both toluene and phenol induced the production of the toluene dioxygenase enzyme and TCE degradation in both strains. Toluene dioxygenase is not the only enzyme responsible for TCE degradation. *Alcaligenes eutrophus* Strain JMP134 uses a phenol hydroxylase enzyme to degrade TCE (Harker and Kim, 1990), and *Pseudomonas cepacia* G4 (G4) expresses a toluene *ortho*-monooxygenase (TOM) enzyme that has been reported to cometabolically degrade chloroethenes. Thus, a variety of phenol- or toluene-oxidizing bacteria in the environment appear to be capable of TCE oxidation, and different mixtures or ratios of those bacteria may be cultivated in mixed biological cultures used for environmental remediation. *P. cepacia* G4 is probably the most widely studied genus of the aromatic TCE-degrading bacteria. The TOM enzyme is inducible by toluene, phenol, and *o*- or *m*-cresol (Nelson et al., 1986; Nelson et al., 1987).

Both phenol- and toluene-oxidizers appear to have similar characteristics to methanotrophs with

regard to product toxicity and competitive inhibition when degrading TCE. However, some phenol degraders have been shown to degrade TCE at higher concentrations without toxic effects, and there have been reports of simultaneous phenol plus TCE degradation (Bielefeldt et al., 1995). Intermediate toxicity was found with *P. cepacia* F1 (Wackett and Householder, 1989) during TCE degradation. TCE oxidation by toluene dioxygenase caused intracellular protein damage, and the cellular toxicity led to reduced growth rates; increasing time of exposure to TCE (from 0 to 9 h) also resulted in increasingly diminished growth rates. However, *P. cepacia* F1 appeared to recover from the toxic effects of TCE oxidation after TCE was removed from the medium.

Bielefeldt et al. (1995) demonstrated that for an inducible, phenol-degrading enrichment, phenol addition enhanced TCE degradation as much as twofold over endogenous degradation rates. The higher rates continued even after phenol was degraded to nondetectable concentrations. Those results suggest that phenol did not significantly compete with TCE and/or that phenol induced the production of dioxygenase enzymes, resulting in increased TCE degradation. The phenol-degrading culture appeared to be uniquely resistant to TCE intermediate toxicity, endowing the culture with unique engineering advantages based on the fact that higher degradation could be obtained in the presence of phenol. Intermediate toxicity was not observed, and the phenol-degrading culture was able to sustain zero-order TCE degradation rates for 30 h, beginning at a TCE concentration of 25 mg/L.

In situ TCE degradation rates reported by Hopkins et al. (1993) increased with increasing phenol concentration, suggesting that TCE- and phenol-oxidizing enzymes were induced by the phenol substrate. When the feed phenol concentration was doubled from 6.2 mg/L to 12.5 mg/L, the dissolved oxygen (DO), *c*-DCE, and TCE concentrations rapidly decreased. By the end of the period with the high phenol addition, a 92% in situ reduction in TCE was observed, along with a 99% destruction of *c*-DCE. TCE removal also increased when *c*-DCE addition stopped, suggesting competitive inhibition between TCE and *c*-DCE. The in situ studies showed that phenol-utilizing bacteria could readily be stimulated in situ. Approximately 3 mg TCE and 3 mg *c*-DCE were removed per g phenol utilized, and 2 mg TCE and 2 mg *c*-DCE were utilized per g DO consumed. For comparison, 5.5 mg *c*-DCE and 1 mg TCE were removed per g methane utilized in a similar experiment, and 2 mg *c*-DCE and 0.4 mg TCE were removed per g DO consumed when methane was the cosubstrate.

Mechanistic differences between the dioxygenase and monooxygenase routes of TCE oxidation may account for differences in TCE intermediate toxicity. For *P. cepacia* G4, TCE is degraded by the toluene *ortho*-monooxygenase enzyme, and relatively little apparent toxicity resulted from TCE metabolism (Folsom et al., 1990); the kinetics of TCE metabolism were linear over 3 h of incubation with a supplied TCE concentration of 2.6 mg/L. In contrast, TCE was toxic to *P. putida* F1 (Wackett and

Gibson, 1988), which utilizes a toluene dioxygenase enzyme. Decreased TCE degradation rates during the course of TCE metabolism were attributed to the formation of toxic by-products during TCE degradation.

TCE and phenol significantly inhibited each other in phenol-grown *P. cepacia* G4 enrichments (Folsom et al., 1990). TCE inhibited phenol degradation in a concentration-dependent manner. The Michaelis-Menten half-saturation coefficient (K_S) is a measurement of the enzyme affinity for a substrate. Lower K_S values indicate increasing enzyme/substrate affinity. Similarly, K_i is a measurement of the affinity of the enzyme for a competing substrate. The ratio of K_i/K_S can be used to estimate relative levels of inhibition between competing substrates. A ratio of K_S to K_i much less than 1.0 would suggest a low level of competitive inhibition by the competing compound on the primary substrate degradation rate. A value of 1.0 or higher would suggest a relatively high level of competition. The data-estimated K_S/K_i ratio was measured for *P. cepacia* G4 grown on phenol (Folsom et al., 1990). Competitive inhibition resulted in reduced phenol degradation rates caused by TCE degradation where the K_S/K_i ratio for phenol and TCE was approximately 1, which is consistent with their similar K_S values (Folsom et al., 1990). The apparent K_S for phenol degradation was 0.8 mg/L and the K_S for TCE degradation was approximately 0.4 mg/L. The maximum phenol degradation rate for *P. cepacia* G4 was 111 mg/mg protein-day and for TCE degradation was 1.9 mg/mg protein-day. Because of the rapid phenol degradation kinetics, phenol was degraded to low concentrations when it was the sole carbon source in continuous culture. At phenol concentrations greater than 3.7 mg/L, phenol transiently inhibited its own degradation, indicating that low phenol (and presumably toluene) concentrations must be maintained to prevent substrate toxicity.

Folsom and Chapman (1991) examined the biodegradation of TCE by *P. cepacia* G4 in a bioreactor. *P. cepacia* G4 was grown in a chemostat with phenol, and TCE was degraded in a separate TCE-degrading reactor that was continuously inoculated with the phenol-grown bacteria and fed TCE. Increased phenol concentrations led to increased biomass production and increased TCE degradation rates, with constant specific TCE degradation rates in the side reactor. The maximum potential for TCE degradation was 1.1 mg/mg protein-day and, on average, the reactor degraded 0.7 mg/mg protein-day. The total amount of TCE degraded increased as either reaction time or biomass was increased in the separate TCE-degrading reactor. TCE degradation was observed at concentrations up to 40 mg/L with no significant decreases in rates. At equal concentrations of TCE and phenol in the TCE-degrading reactor, degradation rates were inhibited 50%, consistent with competitive inhibition and similar K_S values determined for TCE and phenol by Folsom et al. (1990).

Hopkins et al. (1993) demonstrated the use of in situ phenol-degrading microorganisms for TCE degradation. Phenol was fully degraded. The data suggested that competitive inhibition occurred between phenol and *c*-DCE or TCE, and there also were indications that *c*-DCE and *t*-DCE competitively inhibited each other. Cometabolic activity stimulated by phenol addition resulted in higher percent removals of TCE and *c*-DCE than degradation activity stimulated by methane addition, with equal amounts of DO removed. Methane addition resulted in 19 and 43% removal of TCE and *c*-DCE, respectively; phenol addition resulted in 63 and 92% removal, respectively. Removal of TCE per unit of DO consumed for phenol consumption was five times higher than the removal of TCE per unit of DO consumed for methane consumption, indicating that TCE was removed more efficiently by the phenol oxidizers. Those results could suggest that aromatic compounds are more effective cosubstrates, or they could have been due to site-specific conditions and bacteria.

Heald and Jenkins (1994) showed that TCE oxidation resulted in decreased growth rates and caused rapid cell death, which was attributed to intermediate toxicity of TCE oxidation products. However, TCE induced toluene degradation by whole cells to a rate approximately 40% of that by toluene alone, suggesting that TCE could induce the production of toluene monooxygenase enzymes and presumably its own degradation. TCE could not support growth of the organism. TCE was degraded at concentrations as high as 1.3 mg/L; at higher concentrations, the rate of TCE removal declined rapidly, suggesting direct TCE toxicity to the cells or intermediate toxicity via TCE transformation products.

Hecht et al. (1995) investigated the cometabolic degradation of TCE in a 30-L bubble column bioscrubber, using *P. cepacia* G4 cultures and phenol as the cosubstrate and inducer of toluene dioxygenase enzymes required for TCE degradation. Depending on the gas velocity used, degrees of conversion between 30 and 80% were obtained. Degradation of TCE followed pseudo first-order reaction kinetics. No influence of TCE on the steady-state biomass concentration was detected. The reactor showed good long-term stability, having been operated for several months. Hecht et al. (1995) reported rapid TCE mass transfer, and modeling results showed that the process was mainly limited by reaction rate rather than by the mass transfer rate of TCE. Thus, the best way to increase reactor efficiency would be the use of microorganisms with considerably higher degradation rates to overcome the reaction rate limitations, increased reactor volumes, or increased biomass concentrations.

5.1.4 Oxidation of Chlorinated Aliphatic Hydrocarbons by Ammonia-Oxidizing Bacteria

Arciero et al. (1989) were the first to demonstrate that ammonia-oxidizing bacteria could catalyze the degradation of TCE. Suspensions of *Nitrosomonas europaea* were shown to catalyze the

complete disappearance of TCE. However, ammonia oxidation was inhibited 98% in the presence of 1.1 mM TCE. Complete ammonia-oxidizing activity was recovered once TCE was completely degraded. TCE degraded at an initial rate of at least 0.21 mg/mg protein-day. With aged cells, the addition of ammonia appeared to stimulate the rate of TCE degradation.

Three classes of CAC toxicity have been identified according to their inactivating potential (Rasche et al., 1991): (I) compounds that are not biodegraded by *N. europaea* and that have no toxic effect on the cells, (II) compounds that are cooxidized by *N. europaea* and that have little or no toxic effect on the cells, and (III) compounds that are cooxidized and that have significant intermediate toxicity effects on ammonia oxidation by *N. europaea.* Carbon tetrachloride (CT) and PCE, neither of which are capable of being aerobically biodegraded, fall under Class I, suggesting that the solvent toxicity is not caused by direct cell damage by the solvent, but rather by intermediate toxicity via solvent degradation products. Chloromethane, chloroethene, and 1,2-DCA fall under Class II. Class III compounds include DCM and CF; 1,1-DCA, 1,1,1-TCA, 1,1,2-TCA, and 1,1,2,2-TeCA; VC, 1,1-, *c*- and *t*-DCE, and TCE.

Vannelli et al. (1990) also showed that CT and PCE were not degraded by *N. europaea*, but that dibromomethane, vinyl bromide, and *cis*- and *trans*-dibromoethylene were degraded by *N. europaea.* Based on these results and those reported by Rasche et al. (1991), *N. europaea* has a very broad CAC substrate range, similar to methanotrophs.

TCE is a potent competitive inhibitor of ammonia oxidation by *N. europaea* (Hyman et al., 1995). The K_i value for TCE (30 μM) was similar to the K_s value for ammonia (40 μM), and very low ammonia concentrations inhibited TCE degradation. Increasing TCE concentrations led to increasing levels of ammonia inhibition.

5.1.5 Oxidation of Chlorinated Aliphatic Hydrocarbons by Propane-, Propene-, and Isopropylbenzene-Oxidizing Bacteria

Methanotrophs, toluene- and phenol-oxidizing bacteria, and ammonia-oxidizing bacteria represent three groups bacteria that cometabolically degrade TCE and other CACs. Bacteria in the environment oxidize a wide variety of other natural and human-made hydrocarbons. Typically, bacteria initiate their oxidation to grow on hydrocarbons by incorporating oxygen from the atmosphere into organic compounds through the action of enzymes known as oxygenases (Wackett et al., 1989). The oxygenases are generally divided into two groups, the monooxygenases and the dioxygenases. The unique ability of mono- and dioxygenase enzymes to degrade TCE is attributed to their relative

nonspecificity. Many other cosubstrates support bacteria that produce oxygenases capable of TCE degradation, including propane (Wackett et al., 1989; Malachowsky et al., 1994), propylene (Ensign et al., 1992; Reij et al., 1995), and IPB (Dabrock et al., 1992).

Wackett et al. (1989) examined the ability of microorganisms that degrade propane, hexane, cyclohexane, preocene, and nitropropane to degrade TCE. Of the 14 organisms examined, only the 5 propane oxidizers degraded TCE. Those results suggest that TCE oxidation and subsequent biodegradation are not necessarily a general property of oxygenase enzymes and bacteria that contain those enzymes. The propane-oxidizing bacterium *Mycobacterium vaccae* JOB5 degraded all three DCEs and VC, but not PCE. The order of degradation rates were: VC › *c*-DCE › 1,1-DCE › TCE › *t*-DCE.

TCE degradation by two propane-oxidizing *Rhodococcus* species was investigated by Malachowsky et al. (1994). The *Rhodococcus* species degraded TCE and VC with propane as a growth cosubstrate. Cell suspensions degraded TCE at concentrations up to 5 mg/L and VC at concentrations up to 20 mg/L. Propane competitively inhibited TCE degradation; cell suspensions containing 1 mg/L TCE and 40% propane in the headspace degraded 23% of the TCE, compared to 87% TCE degradation without propane. Cell suspensions degraded up to 5 mg/L of TCE and up to 40 mg/L of VC. Propane-grown resting cell suspensions also degraded chloroform (CF), 1,1-DCE, *c*-DCE, and 1,1,1- and 1,1,2-TCA, suggesting that propane-oxidizing bacteria can degrade a wide variety of different CACs, similar to methanotrophs and ammonia-oxidizing bacteria. PCE, CT, and *t*-DCE were not degraded by the propane oxidizers. The *Rhodococcus* isolates also degraded benzene, toluene, sodium benzoate, naphthalene, biphenyl, and *n*-alkanes ranging in size from propane to hexadecane as carbon and energy sources.

Propylene also can serve as a suitable cosubstrate for TCE degradation. Propylene-grown *Xanthobacter* Py2 cells degrade TCE, but the transformation capacity may be limited and depends on both the TCE and the biomass concentrations (Reij et al., 1995) . The affinity of Strain Py2 for TCE was low (i.e., a high K_S), which allowed it to grow on propylene while degrading TCE, but also resulted in competition between propylene and TCE and thus in reduced TCE degradation rates in the presence of propylene. TCE degradation was inhibited by high propylene concentrations and did not start before most of the propylene had been consumed. Propylene degradation rates also were inhibited by the presence of TCE. In spite of the competitive inhibition of propylene on TCE degradation, low propylene concentrations can stimulate CAC degradation, although at high concentrations propylene becomes extremely inhibitory (Ensign et al., 1992).

Propylene-grown *Xanthobacter* strains degrade TCE, VC, *c*- and *t*-DCE, 1,3-DCP, and 2,3-DCP, whereas 1,1-DCE and PCE are not degraded (Ensign et al., 1992). *Xanthobacter* is subject to some of

the same toxicity problems as described for methane-, toluene-, phenol-, ammonia-, and propane-oxidizing bacteria. However, Reij et al. (1995) reported that biomass yields on propylene were not affected by the cometabolic degradation of TCE, suggesting that TCE degradation was not toxic to the *Xanthobacter* propylene degraders, or that they gained energy from the oxidation of TCE degradation products.

Bacteria grown on IPB also demonstrated CAC degradation abilities (Dabrock et al., 1992). IPB-, phenol-, toluene-, citronellol-, and dipentene-grown cells were tested for their ability to cometabolically degrade TCE; 27 out of 27 IPB-grown strains tested positive for TCE degradation, compared to 13 out of 27 toluene-degrading strains, 2 out of 9 phenol-degrading strains, and zero out of 4 citronellol- and 11 dipentene-degrading strains, respectively, indicating that a high proportion of IPB-degrading bacteria can degrade TCE. *Rhodococcus erythropolis* BD1 degrades TCE and *c*- and *t*-DCE with IPB as a primary growth substrate, whereas *Pseudomonas* species Strain JR1 degrades all three DCEs, VC, 1,1,2-TCA, and 1,2-DCA (Dabrock et al., 1992). Dabrock et al. (1992) showed that TCE degradation rates with IPB-oxidizing bacteria increased with increasing initial TCE concentrations, and a linear dependence was observed between 0 and 200 μM. The time after which the activity ceased appeared to be concentration-independent, and the apparent inactivation did not appear to be a result of toxic TCE effects because it was overcome by substrate refeeding. Thus, similar to some phenol-oxidizing cultures described above, the IPB-oxidizing bacteria appear to be resistant to intermediate TCE toxicity. The maximum initial TCE degradation rate for *R. erythropolis* BD1 was 1.2 mg/mg protein-day, which was comparable to the TCE degradation rate for *P. cepacia* G4 (Folsom et al., 1990).

5.1.6 Summary of Cometabolism

Several cosubstrates have been implicated in the cometabolic degradation of CACs. They include methane, ammonia, propane, propylene, and aromatic compounds such as toluene, phenol, and IPB. Table 6 shows advantages and disadvantages of each cosubstrate. A common disadvantage of all the cosubstrates is the intermediate toxicity that occurs during TCE oxidation. However, some phenol- and IPB-degrading bacteria appear to be able to degrade TCE at higher concentrations without toxic effects. Bielefeldt et al. (1995) reported simultaneous phenol plus TCE degradation without intermediate toxicity, and relatively little apparent toxicity results from TCE metabolism by *P. cepacia* G4 (Folsom et al., 1990). Dabrock et al. (1992) reported that the IPB degraders also were not affected by intermediate toxicity; however, no data were presented supporting their argument. In contrast, there are no reports of methanotrophs, ammonia oxidizers, or propane oxidizers that are resistant to intermediate toxicity.

TABLE 6. ADVANTAGES AND DISADVANTAGES OF DIFFERENT COSUBSTRATE-OXIDIZING BACTERIA FOR COMETABOLIC CAC DEGRADATION

Cosubstrate	Advantages	Disadvantages
Methane	Methanotrophs degrade a wide variety of CACs Formate can supply additional energy Extensive body of literature available	Competitive inhibition Intermediate toxicity Requires low aqueous Cu^{2+} concentrations Low solubility Explosive Low proportion of CH_4-oxidizers contribute to CAC degradation
Toluene/Phenol	High solubility Rapid growth Simple process control Concurrent phenol- and CAC-oxidation is possible Selected cultures do not exhibit intermediate toxicity, and those strains may be resistant to intermediate toxicity Extensive body of literature available	Phenol-oxidizers do not degrade chloroethanes Competitive inhibition Low proportion of phenol-oxidizers contribute to CAC degradation
Ammonia	Ammonia-oxidizers degrade a wide variety of CACs Cells can recover from intermediate toxicity in the absence of CACs High solubility Inexpensive	Severe competitive inhibition Intermediate toxicity Produces nitrate Limited literature information available Low proportion of ammonia-oxidizers contribute to CAC degradation
Propane	Possibly degrade a wide variety of CACs	Low solubility Explosive Competitive inhibition Intermediate toxicity Limited body of literature available
Propylene	High solubility Low levels of intermediate toxicity	Propylene-oxidizers do not degrade chloroethanes Competitive inhibition Limited body of literature available
Isopropylbenzene (IPB) (cumene)	Degrade a wide variety of CACs High solubility Simple process control Possibly low competitive inhibition High percentage of IPB-oxidizers may degrade CACs Some strains may be resistant to intermediate toxicity	Low solubility Explosive Competitive inhibition Intermediate toxicity Limited body of literature available

Another common disadvantage of cometabolic CAC degradation is competitive inhibition between the cosubstrate and TCE (and presumably among the CACs themselves). Competitive inhibition has been reported for nearly all of the cosubstrates investigated. However, competition results in different degrees of inhibition of TCE degradation, depending on the species of bacteria and its relative affinities for TCE and the cosubstrate. For example, the ammonia oxidizers appear to be among the most severely inhibited bacteria with respect to TCE degradation, where only very low ammonia concentrations can result in significantly reduced TCE degradation rates. Methane, phenol, and toluene inhibit TCE degradation to varying degrees.

An important feature of the methanotrophs, the ammonia oxidizers, and the propane oxidizers is their ability to degrade chloroethanes in addition to TCE and other chloroethenes. Of the aromatic-degrading bacteria, only the IPB degraders were reported to degrade both chloroethenes and chloroethanes. The phenol- and toluene-degrading bacteria repeatedly have been shown not to degrade chloroethanes.

Other important features of the different cosubstrates are whether they are explosive, their aqueous solubilities, the body of literature supporting their use, the proportion of cosubstrate-oxidizing bacteria that directly contribute toward CAC degradation, and bulk costs. Methane and propane are explosive and have very low aqueous solubilities, which would necessitate their application at high gas-phase concentrations. Phenol, toluene, and IPB are not explosive and have much higher aqueous solubilities, but they are more toxic to bacteria at higher concentrations than the other cosubstrates, and they require more careful process control to ensure that they are completely degraded. The number of cosubstrate-oxidizers that contribute toward CAC degradation will impact the cost of their application. For example, if a low proportion of methanotrophs in a methane-fed reactor degrades TCE, than a large fraction of the methane added to the system will not contribute toward TCE degradation, resulting in inefficient methane use. Dabrock et al. (1992) investigated 27 different IPB-degrading strains of bacteria, and all 27 degraded TCE. Only 13 out of 27 toluene-degraders and 2 out of 9 phenol-degraders degraded TCE. These results suggest that not all of the toluene- and phenol-degrading bacteria can degrade TCE, but a high percentage (possibly 100%) of IPB-degrading bacteria appear to degrade TCE. That would make IPB a relatively efficient cosubstrate for TCE degradation.

Table 7 summarizes specific TCE degradation rates and concentration tolerances of cosubstrate-degrading enrichments (modified from Bielefeldt et al., 1995) for comparison. It is difficult to make a specific comparison of all the cultures because of their different growth and environmental conditions. In addition, pure cultures are expected to have much higher specific growth rates than mixed cultures because a much higher percentage of the normalized biomass concentration contributes directly to TCE

TABLE 7. REPORTED MAXIMUM TCE DEGRADATION RATES AND TCE CONCENTRATIONS TESTED FOR DIFFERENT COSUBSTRATE-OXIDIZING CULTURES

Culture	Cosubstrate	Reported TCE degradation rate (g/g VSS-d)	Highest TCE concentration degraded/tested (mg/L)	Test Temp (°C)	Reference
OB3b	Methane	1.58-3.78	10.6	30	Tsien et al. (1989)
Mixed methanotroph	Methane	0.58 - 2.0	21	21	Alvarez-Cohen and McCarty (1991a)
Mixed methanotroph	Methane	0.015			Phelps et al. (1990)
Mixed methanotroph	Methane	0.021-0.026	15	28	McFarland et al. (1992)
Mixed methanotroph	Methane	0.027	7.8	20	Strand et al. (1990)
P. putida F39/D	Phenol	0.62	› 9.4	30	Zylstra et al. (1989)
P. putida	Toluene	1.2	1.6	30	Heald and Jenkins (1994)
P. cepacia G4	Phenol		» 70, ‹ 428	23	Shields and Reagin (1992)
P. cepacia G4 PR1	Phenol	0.09	› 70, ‹ 428	23	Shields and Reagin (1992)
P. cepacia G4	Phenol	1.1 (0.7)	50	28	Folsom and Chapman (1991)
P. cepacia G4	Phenol	1.92	30	26	Folsom et al. (1990)
P. cepacia G4	Toluene	0.12-0.18		28	Landa et al. (1994)
Filamentous enrichment	Phenol	0.10-0.25 (0.18)	130	20	Bielefeldt et al. (1995)
A. eutrophus JMP134	Phenol	0.05	-	30	Harker and Kim (1990)
N. europaea	Ammonia	0.21	1.4	20	Arciero et al. (1989)
M. vaccae JOB5	Propane	0.07	4.15	--	Wackett et al. (1989)
Xanthobacter PY2	Propylene	0.36	41.5	30	Reij et al. (1995)
R. erythropolis BD1	Isopropylbenzene IPB (cumene)	1.2	33	20	Dabrock et al. (1992)

degradation in pure cultures. In general, the phenol-degrading enrichments reported the highest endogenous transformation capacities (Tc), which is a measurement of the observed mass of TCE degraded per mass of protein inactivated by TCE degradation. The degradation rates can be used to determine relative differences in the abilities of different cosubstrate-degrading bacteria to degrade CACs.

The highest concentration degraded, shown in Table 7, more frequently represents the maximum TCE concentration tested, rather than a toxicity threshold. The fact that the tested TCE concentrations often exceeded the projected total aqueous CAC concentration from the SVE off-gas suggests that CACs will not be present at toxic concentrations in the reactor(s).

Table 8 shows the cosubstrate transformation capacities of TCE using different cosubstrate-oxidizing cultures, measured as the cosubstrate consumed per mass of TCE degraded. A wide range of transformation capacities has been reported, suggesting that the amount of cosubstrate required for CAC degradation is culture-specific, and it may depend upon specific experimental and environmental conditions. Thus, the optimal amount of cosubstrate required for CAC degradation in the SVE off-gas will have to be determined experimentally in the laboratory and field demonstrations. For a steady-state system, optimal cosubstrate concentrations must be controlled so that they can support a cosubstrate-degrading population large enough to degrade the CACs to predetermined effluent concentrations, but low enough to limit or prevent competitive inhibition between the cosubstrate and the CACs. Lower cosubstrate concentrations also will result in reduced cosubstrate costs during full-scale operation.

TABLE 8. REPORTED TCE TRANSFORMATION CAPACITIES OF DIFFERENT COSUBSTRATES

Culture	Cosubstrate	Transformation Capacity (g cosubstrate consumed per g of TCE degraded)	Reference
Soil microcosm	Methane	1,000	Hopkins et al. (1993a)
M. trichosporium Ob3b	Methane	320-1,200	Oldenhuis (1992)[a]
Mixed culture	Methane	77	Alvarez-Cohen and McCarty (1991a)
Mixed culture	Methane	11-30	Phelps et al. (1990)
Soil microcosm	Phenol	9	Hopkins et al. (1993a)
	Phenol	50	Folsom and Chapman (1991)
Soil, in situ	Phenol	500	Hopkins et al. (1993b)
P. cepacia G4	Toluene	14-71	Landa et al. (1994)
P. cepacia G4	Phenol	2-10	Ensley (1992)
P. cepacia G4	Toluene	4-40	Ensley (1992)
N. europaea	Ammonia	1.6	Arciero et al. (1989)
Xanthobacter PY2	Propylene	4-23	Reij et al. (1995)

[a] As cited in Reij et al., 1995.

5.1.7 Direct CAC Degradation

Most bacteria capable of cometabolic TCE degradation also are able to cometabolically degrade DCEs and VC. However, VC appears to be unique among the chloroethenes in its ability to be degraded as a sole carbon and energy source (Hartmans and de Bont, 1992). *Mycobacterium aurum* L1 grows on VC as a sole carbon and energy source. Hartmans and de Bont reported that three additional strains similar to Strain L1 and identified as *M. aurum* grew on VC as a sole carbon and energy source. The rate of VC degradation was 5 mg/mg cells-day. *M. aurum* L1 also oxidized dichloroethenes at rates that were in the same range as VC; 1,1-DCE was degraded at 1.4 mg/mg cells-day, *c*-DCE was degraded at 4.2 mg/mg cells-day, and *t*-DCE was degraded at 3.5 mg/mg cells-day. The authors did not report whether the DCE degradation contributed carbon and energy toward cell growth, and TCE was not degraded.

Several organisms have been shown to grow on 1,2-DCA as a sole carbon and energy source (Janssen et al., 1985; Strotmann et al., 1990; van der Ploeg et al., 1994). However, similar to chloroethenes, the higher chlorinated ethanes are degraded only cometabolically under aerobic conditions. Organisms capable of utilizing CACs for growth can be used for the removal of these

substrates from wastestreams such as the contaminated SVE off-gas at McClellan AFB.

5.1.8 Degradation of Additional Constituents: Dichlorobenzenes, BTEX, and Acetone

Pure cultures of *Pseudomonas* (Haigler et al., 1988; Spain and Nishino, 1987) and *Alcaligenes* (de Bont et al., 1986; Schraa et al., 1986) and *Xanthobacter* strains (Spiess et al., 1995), which use DCBs as the sole source of carbon and energy, have been shown. Each species appears to attack DCBs by a dioxygenase, producing chlorocatechols that are degraded subsequently. The initial oxidation of DCBs appears to be the rate limiting step toward their degradation (Schraa et al., 1986); and growth rates are generally slow, requiring large bioreactor volumes or long solids retention times. Long solids retention times can be provided by a fixed-film biological process, which retains biomass longer than complete-mix systems.

In general, BTEX compounds and acetone readily degrade in aerobic biological systems. Their degradation rates are very fast, and they can be degraded efficiently to relatively low concentrations in biological systems (Wiedemeier et al., 1995; Bielefeldt et al., 1995). Because BTEX and acetone are easily and rapidly degraded, they are expected to be among the first compounds to be degraded in the contaminated off-gas stream, followed by increasingly recalcitrant compounds.

5.1.9 Anaerobic Reductive Dechlorination

Many of the halogenated compounds discussed above are relatively recalcitrant to aerobic degradation. As a general rule, increasing the number of chlorines on a carbon-based molecule renders it more difficult to degrade aerobically. The opposite can be said under anaerobic conditions. Under anaerobic conditions, the more-chlorinated compounds tend to be dechlorinated more rapidly than the less-chlorinated compounds, possibly because they are in a more oxidized state due to the chlorine halogens. Thus, the order of chloroethene degradation rates is PCE › TCE › DCE » VC. Usually, VC is the rate-limiting step for complete PCE dechlorination to ethene, and it tends to accumulate in the environment or in biological systems. Similarly, higher-chlorinated benzene compounds tend to be more easily dechlorinated under anaerobic conditions than the lower chlorinated benzenes, such as chlorobenzene and most DCBs.

Anaerobic dechlorination has been well established for chloroethenes and chloroaromatic compounds. Less information is available for chloroethanes, although they too can be dechlorinated anaerobically. The chloroethenes are dechlorinated via a relatively simple pathway that involves the

step-wise removal of chlorines to form ethene as the final product. Unlike the chloroethenes, chloroethanes can be dehalogenated to lower chloroethanes or they can be degraded biologically or abiotically to lower chlorinated ethenes (Chen et al., 1996; Vogel et al., 1987), which involves the transformation of the carbon-carbon bond to a double bond during dechlorination. 1,1,1-TCA transformations to 1,1-DCA (Gälli and McCarty, 1989) and 1,1-DCE have been reported (Vogel et al., 1987), and 1,1,2-TCA can be transformed to 1,2-DCA or VC (Chen et al., 1996).

Enzien et al. (1994) reported the reductive dechlorination of PCE and TCE under bulk aerobic conditions in a sediment column. The reductive dechlorination was attributed to micro anaerobic communities in the aerobic sediment column, suggesting the potential for simultaneous aerobic and anaerobic biotransformation processes under bulk aerobic conditions. Methane production also contributed to the conclusion that anaerobic activity was present in the sediment column. PCE and TCE were dechlorinated to *c*-DCE which showed no sign of being dechlorinated to VC.

6.0 VAPOR-PHASE BIOLOGICAL REACTOR TECHNOLOGY

The treatment of contaminated vapors (i.e., odors, VOCs, and toxic air pollutants) has received increased attention in recent years, largely as a consequence of the 1990 Clean Air Act Amendments (CAAA). The CAAA require a 90% reduction in specific hazardous air pollutants (HAPs) released from major emission sources by the year 2000 (Zahodiakin, 1990). Currently, 189 HAPs are targeted for reduction under Title III of the CAAA. In addition, Title I of the CAAA requires a 15% reduction of *all* VOCs and nitrogen oxides (NO_X) in areas of the country with especially high ground ozone levels (ozone nonattainment areas) by the year 1996 (Begley, 1991). VOCs effect the nitrogen dioxide (NO_2) photolytic cycle, and also contribute to the formation of ground-level ozone and other oxidants, the major components of photochemical smog (Wark and Warner, 1981).

The available options for VOC and HAP reduction from individual sources include (1) process changes, (2) raw materials reformulation, and/or (3) installation of point source control measures. Existing point source control options include (1) wet scrubbing, (2) carbon adsorption, (3) thermal oxidation, (4) carbon adsorption, (5) catalytic oxidation, and (6) biological treatment. It is estimated that expenditures on emission controls required by the CAAA could reach $50 billion by the year 2005 (Heller, 1991).

Biotreatment of contaminated vapors is a relatively recent development in the United States. Traditional vapor scrubbing, thermal incineration, catalytic incineration, and adsorption to activated carbon have all been used to treat airborne contaminants in the past. However, all these methods are

potentially more expensive than biotreatment (Chetty et al., 1992; Dharmavaram, 1991). In addition to economic issues, another drawback of both traditional vapor scrubbing and adsorption to activated carbon is that these methods do not destroy the toxic contaminants of interest, but merely transfer them from one medium (air) to another (i.e., liquid or solid) medium. Further processing is necessary to destroy the contaminants. Biotreatment processes are environmentally friendly, and they produce only nonhazardous byproducts such as additional biomass, water, and low levels of carbon dioxide. No carbon monoxide, NO_x, SO_x, or thermal pollution is produced.

6.1 Conventional Biofilters

One type of biological air treatment process commercially available is biofiltration (Leson and Winer, 1991; van Groenestijn and Hesselink, 1993; VDI Richtlinie 3477, 1991). Biofiltration is a process that utilizes microorganisms immobilized in the form of a biofilm layer on a porous, absorbent filter packing material. As a contaminated vapor stream passes through the filter bed, pollutants are transferred from the vapor to the biolayer and are oxidized, forming carbon dioxide and water, or, in the case of odors, are transformed into less- or nonodorous compounds. The packing material used in conventional biofilters is usually composed of compost, wood chips, peat, heather, or combinations of these materials (Leson and Winer, 1991; VDI Richtlinie 3477, 1991). In addition, bulking agents, such as polystyrene beads, and pH buffering agents such as calcium carbonate or lime, can be added to enhance the mechanical and performance properties of the packing (van Lith et al., 1990). Activated carbon has also been investigated as a packing material for petroleum hydrocarbon and ethanol applications (Hodge et al., 1991; Hodge and Devinny, 1994; Devinny and Hodge, 1995).

The simplest form of biofiltration system is the soil bed, where a horizontal network of perforated pipe is placed 2 to 3 feet below the ground (Kampbell et al., 1987; Bohn and Bohn, 1988; Bohn, 1992). Vapor contaminants are pumped through the piping, flow upward through the soil pores, and are oxidized by microorganisms present within the soil. However, efficient and reliable biofiltration requires a much more controlled environment than typically found within soil beds. Control of temperature, bed moisture content, and pH is necessary if the microorganisms responsible for biodegradation are to function efficiently. The need for a controlled environment has led to the development of sophisticated units containing better defined filter support and organisms cultured within the laboratory (van Lith et al., 1990; Togna et al., 1993). Biofilters are most economical for treating high-volume air flows (1,000 to 100,000 cfm and above) containing low concentrations (less than 1,000 ppmv) of biodegradable organic compounds (Dyer and Mulholland, 1994; Vembu and Walker, 1995).

To prevent the biofilter bed from drying out, the influent vapor stream usually is prehumidified to as close to 100% water saturation as possible. Water typically is added directly to the packing material to replace water lost from the packing due to the exothermic nature of the biological oxidation process (van Lith et al., 1990). Both upflow (countercurrent) and downflow (cocurrent) configurations have been used.

Biofiltration has been used in Europe for over 30 years to control odorous air emissions (Leson and Winer, 1991). Biofilters have also been used in the United States to treat hydrogen sulfide, mercaptans, alcohols, and other odor-causing airborne contaminants emitted from wastewater treatment plants, industrial process streams, and composting facilities (Allen and Yang, 1992; Yang and Allen, 1994a; Yang and Allen, 1994b; Leson et al., 1993; Kuter et al., 1993). Recent developments in biofilter technology have expanded the range of treatable target compounds to include a wide range of VOC air pollutants (Leson and Winer, 1991; Fouhy, 1992; Togna et al., 1993; Ergas et al., 1993; Yavorsky, 1993; Langseth and Pflum, 1994; Lacky and Holt, 1996). One such advance has been the development of biofilters to treat petroleum hydrocarbon vapors (Peters et al., 1993; Zurlinden et al., 1994; Hodge et al., 1991; Apel et al., 1993; Togna et al., 1994; Leson and Smith, 1995). In cases where off-gases are produced during vapor extraction operations containing low to moderate concentrations of hydrocarbons, biofiltration has the potential to be a cost-effective alternative to conventional air treatment technologies such as incineration and carbon adsorption.

Biofiltration systems have been investigated for the removal of TCE and other chlorinated compounds from wastewater treatment plant emissions (Ergas et al., 1992; Webster et al., 1995). Typically, these compounds are present at very low concentrations (100 ppbv) within these streams (Ergas et al., 1992). For these applications, the biofilter systems usually rely on trace levels of aromatic compounds or methane in the vapor stream to act as cosubstrates for TCE removal. TCE DREs typically are only 50% at economically viable reactor vapor contact times (Ergas et al., 1992; Webster et al., 1995), where the vapor contact time is defined as the reactor volume occupied by packing divided by the vapor flowrate.

Biofilters have also been investigated for removal of TCE at higher concentrations (10 to 50 ppmv) using methane or propane as cosubstrates (Griffiths et al., 1995; HazTech News, 1996). In one test, 80% TCE removal could be attained for a short period of time (9 days) after pulsing with propane, but only at a very long vapor contact time of 55 minutes (Griffiths et al., 1995). During normal operation, at a steady propane or methane feed, the TCE removal efficiency was approximately 50%. In another test, greater than 90% TCE removal could be attained at a 30-minute vapor contact time (HazTech News, 1996). This test also used propane as the cosubstrate.

6.2 Fixed-Film Trickling Filters (Biotrickling Filters)

A second type of biological system used to treat gas-phase VOCs is the biotrickling filter. Biotrickling filters are similar to biofilters, but contain a stable, solid packing material instead of compost or peat, and operate with liquid medium flow over the packing to facilitate mass transfer (Dharmavaram, 1991; Hartmans and Tramper, 1991; Togna and Folsom, 1992; van Groenestijn and Hesselink, 1993; Togna and Singh, 1994). Only the recirculating liquid is inoculated initially with microorganisms, but a biofilm layer forms on the packing shortly after startup. Contaminants are transferred to, and degraded by, microorganisms present within both the recirculating liquid and the biofilm layer, although the majority of the degradation is performed within the biofilm. Biotrickling filters can be operated in either an upflow mode, countercurrent to the flow of the recirculating medium, or downflow mode, in a downflow mode, cocurrent to the flow of the recirculating medium. However, the downflow mode of operation has been shown through mathematical modeling to result in higher removal efficiencies for less water-soluble contaminants because absorbed contaminants will not be restripped from the recirculating water during this mode of operation (Ockeloen et al., 1992). Previously, it had been thought that biotrickling filters were not capable of effectively treating sparingly soluble contaminants with Henry's law coefficients greater than 0.02 atm-m^3/mol (van Groenestijn and Hesselink, 1993). However, it has been shown recently that biotrickling filters are more effective than biofilters for treatment of isopentane, which has a Henry's law coefficient of 1.3 atm-m^3/mol, possibly due to the adsorptive influence of the microbial biomass (Togna and Singh, 1994).

Biotrickling filters have some distinct process advantages over conventional biofilters. First, the pH of the recirculating liquid within biotrickling filters is easily monitored and controlled by the automatic addition of acid or base. The pH within conventional biofilters is controlled by the addition of solid calcium carbonate or lime to the packing material at the beginning of operation (van Lith et al., 1990). Once the buffering capacity is exhausted, and the pH of the bed material drops, the filter bed is removed and replaced with fresh material. For the treatment of air streams containing halogenated or sulfur-containing contaminants that generate acids upon biodegradation, biofilter bed replacement can be quite frequent (Ergas et al., 1995). Therefore, biotrickling filters are frequently more cost effective than biofilters for treating halogenated contaminants such as TCE and methylene chloride, or sulfur compounds such as hydrogen sulfide (H_2S) and carbon disulfide (CS_2) (Hartmans and Tramper, 1991; Diks and Ottengraf, 1991a; Diks and Ottengraf, 1991b; van Lith et al., 1993). However, in cases where only H_2S and/or CS_2 are/is present, biofilters have been shown to be capable of sustained contaminant

removal under low pH conditions (Yang et al., 1993a; Yang et al., 1993b; Yang et al., 1994; Yang and Alibeckoff, 1995). Second, biotrickling filters allow for much greater control of process parameters, including salt removal and nutrient/supplemental food addition, and therefore are much more flexible and durable than biofilters. This is especially important for treatment of air streams containing recalcitrant contaminants such as TCE that can be treated only by adding a cosubstrate to the system. Both biofilters and biotrickling filters can be used to treat air streams containing TCE using methanotrophs by feeding methane to the air stream. However, liquid cosubstrates (such as phenol and toluene) and sources of reducing equivalents (such as formate) are more easily added to biotrickling filters than to biofilters. Finally, biotrickling filters may offer higher removal efficiencies at higher organic loadings than biofilters, possibly due to thicker biofilm development or the adsorptive influence of the microbial biofilm (Togna and Folsom, 1992; Togna and Singh, 1994).

Biotrickling filters also offer space advantages compared to conventional biofilters. The packing height in conventional biofilters is usually limited to 4 feet due to problems of bed compaction over time. Therefore, biofilters tend to occupy a significant area. Biotrickling filters, however, often show significantly better performance than biofilters (Togna and Folsom, 1992; Togna and Singh, 1994), and therefore can be designed as smaller skid-mounted units, allowing for much more efficient use of space. In addition, biotrickling filters contain inert packing materials that are not subject to deterioration and compaction. Therefore, packing replacement is not required, and the systems can be designed as columns. Packing materials typically used within biotrickling filters include corrugated plastic packings (Togna et al., 1995; Torres-Cardona et al., 1993), dumped plastic and metal scrubber packing (Hartmans and Tramper, 1991; Pedersen and Arvin, 1995), carbon-impregnated polyurethane foam (DeFilippi et al., 1993), structured ceramic packing (Govind et al., 1993), activated carbon (Govind et al., 1993), Celite® (Sorial et al., 1994; Sorial et al., 1995), and even oyster shells (Shields et al., 1993).

Considerable attention has been devoted recently to biomass growth-control mechanisms within biotrickling filters. These mechanisms primarily involve operational/biological control methods. Three general control methods have been investigated. These are (1) limiting an inorganic nutrient such as nitrogen (Weber and Hartmans, 1994) or potassium (Holubar et al., 1995), (2) increasing the ionic strength (Weber and Hartmans, 1994) or salt (NaCl) concentration (van Lith et al., 1994; Diks et al., 1994a), and (3) incorporating mechanical liquid/vapor shear mechanisms (Togna et al., 1995) or backwashing (Sorial et al., 1994; Sorial et al., 1995). However, nitrogen limitations have been shown to significantly decrease bioreactor performance (Holubar et al., 1995). Diks et al. (1994b) recently established a steady-state biomass concentration within a biotrickling filter treating methylene chloride,

where almost all of the methylene chloride carbon was converted to carbon dioxide. This steady-state biomass concentration in the biotrickling filter was attributed to rapid endogenous respiration and/or predation by protozoa that countered the growth of methylene chloride degrading bacteria (Diks et al., 1994b).

Recent field-pilot and full-scale biotrickling filter applications include treatment of (1) methylene chloride emissions from an artificial glass production process (van Lith et al., 1993); (2) N, N-dimethylacetamide emissions released during production of Lycra® fibers (Dharmavaram et al., 1995); (3) acetone and ethanol emissions from a cosmetic pencil production process (Loy, 1995); (4) ethanol and ethyl acetate emissions from a printing operation (Loy, 1995); (5) odorous vapor emissions released during the production of tobacco and compost (Loy, 1995); (6) hydrogen sulfide and carbon disulfide emissions from cellophane, rayon, and sponge production processes (Revah et al., 1994; Revah et al., 1995); (7) ammonia emissions from a composting operation (Smits et al., 1995); (8) isopentane and isobutane emissions from a foam manufacturing facility (Togna et al., 1995); and (9) wastewater treatment plant emissions, including low concentrations of chlorinated VOCs (Envirogen, unpublished data).

Biotrickling filter systems have been investigated for removal of TCE from air streams (Speitel and McClay, 1993; Shields et al., 1993; Govind et al., 1993; Govind et al., 1995). Speitel and McClay (1993) demonstrated 20 to 80% removal of TCE at packed-bed vapor contact times of 5 to 12 min using methane as a cosubstrate and the bacterium *Methylosinus trichosporium* OB3b. The TCE concentration range investigated by Speitel and McClay was between 300 and 1,000 μg/L (55 and 190 ppmv). Shields et al. (1993) investigated removal of TCE from air using a constitutive mutant of *P. cepacia* G4. The packing material utilized by Shields et al. was composed of oyster shells. A nutrient mineral solution containing yeast extract, peptone, glucose, and lactic acid was continuously recirculated through the column. After a 48 h inoculation period, 90% TCE removal was demonstrated over a 4-day period at an inlet TCE concentration of 130 μg/L (25 ppmv) and a 4-h vapor contact time (Shields et al., 1993). Govind et al. (1993) demonstrated sustained TCE removal from a mixed wastestream using a biotrickling filter containing activated carbon pellets as the packing material. Toluene within the vapor stream was utilized as the cosubstrate at a concentration of 520 ppmv. Govind et al. (1993) demonstrated sustained TCE and toluene removal efficiencies of greater than 95% for over 3 months at vapor contact times between 2 and 4 min and an inlet TCE concentration of 25 ppmv. However, when a ceramic packing material was used, only 30 to 40% TCE removal was observed (Govind et al., 1993). This difference in performance was attributed to adsorption of the cosubstrate (toluene) onto the carbon

packing, which allowed for a low level of toluene usage by the TCE-degrading microorganisms in sections of the column where very little toluene remained in the vapor. In the system containing the ceramic packing, toluene was removed almost completely at the entrance of the column, and little toluene was available in the other portions of the column for TCE removal. More recently, Govind et al. (1995) have demonstrated removal of TCE and PCE within a biotrickling filter using microorganisms encapsulated within a hydrogel. The removal of TCE and PCE was attributed to partial reductive dechlorination caused by anaerobic conditions within the interior of the hydrogel created by consumption of oxygen at the surface. Greater than 99% TCE removal was demonstrated at a vapor contact time of 1.2 min for an inlet TCE concentration of 25 ppmv and an inlet toluene concentration up to 100 ppmv. Greater than 99% PCE removal was demonstrated at a vapor contact time of 4 min for an inlet PCE concentration of 25 ppmv and an inlet toluene concentration up to 100 ppmv (Govind et al., 1995).

6.3 Bioscrubbers

A third type of biological system used for treating contaminated vapor streams is the bioscrubber. Bioscrubbing is a process whereby absorption of vapor-phase contaminants into an aqueous phase occurs, with the subsequent oxidation of the contaminants by microorganisms suspended in the liquid (Dharmavaram, 1991; van Groenestijn and Hesselink, 1993; VDI Richtlinie 3478, 1985).

For most bioscrubbing systems, absorption and biological oxidation are separated into two distinct unit operations, with absorption of the contaminants into water occurring in a scrubber column, followed by the degradation of the contaminant in a biological reactor (Dharmavaram, 1991; van Groenestijn and Hesselink, 1993; VDI Richtlinie 3478, 1985; Overcamp et al., 1993). The biological reactor is frequently an activated sludge system (VDI Richtlinie 3478). Using mathematical modeling, Overcamp et al. (1992) have found that bioscrubbers are very economical for degrading highly soluble contaminants, such as alcohols, whereas biotrickling filters should be used for treating less-water-soluble contaminants. However, the use of activated carbon in the recirculating water has recently been proposed as a way of enhancing the solubility and degradability of sparingly soluble contaminants (Overcamp et al., 1994; Kok, 1994). Bioscrubbers typically have been used for treating odors, but a recent bioscrubbing application includes treating ethanol emissions from a brewery (Croonenberghs et al., 1994).

Envirogen also has demonstrated that bioscrubbers can be designed so that absorption into water and biological oxidation occur concurrently in the same reactor. By designing the system in this manner, the consumption of the contaminant in the liquid droplets by the microorganisms can be used to increase

the mass transfer rate into the droplets. In addition, mass transfer appears to be enhanced by small liquid droplets due to adsorption effects. It has been observed that "fog," or small aqueous droplets 10 μm or less in diameter, absorb vapor contaminants with much higher efficiency, sometimes two or four orders of magnitude higher, than would be predicted based on Henry's law alone (Capel et al., 1991; Glotfeldy et al., 1990; Glotfeldy et al., 1987).

6.4 Suspended-Growth Reactors

A fourth type of biological system used for treatment of contaminated vapor streams is the suspended-growth reactor (Dasu et al., 1993; Ensley and Kurisko, 1994). These reactors may be bubble columns or stirred tanks, and may even contain activated carbon as a biomass support matrix (Ye et al., 1994). These systems are most effective when a very high degree of process control is required, and are most economical for treatment of low-volumetric-vapor flowrates (100 to 200 cfm) containing high concentrations of contaminants (greater than 1,000 ppmv as C) (Radian/Envirogen, 1996; Envirogen, 1996).

Greater than 90% TCE removal has been demonstrated using the bubble column design when treating a TCE/benzene stream (Folsom, 1992), when treating a wastestream containing other chlorinated solvents (DOE report no. DOE/OR/21400-T492), and when treating a pure TCE wastestream at concentrations 5 to10 mg/L of TCE in the vapor phase (Ensley, 1992). Effective long-term performance for over 9 months, averaging over 90% TCE removal, was demonstrated with the stirred-tank design (Envirogen, 1996). In these studies, *P. cepacia* strain G4 and/or *P. mendocina* were used along with phenol (for *P. cepacia* G4) and/or toluene (for both *P. cepacia* and *P. mendocina*) as cosubstrates for TCE degradation. The stirred-tank reactor design and TCE treatment process have been effectively demonstrated in the field at Robins AFB, Georgia, and at F.E. Warren AFB, Wyoming using a 1,000-gallon tank (Envirogen, 1996; Radian/Envirogen, 1996). At F.E. Warren AFB, 85 to 90% total TCE removal was demonstrated over a 70-day period (Radian/Envirogen, 1996).

6.5 Membrane Bioreactors

Membrane biofilters employ semi-permeable membranes that are impermeable to water. The biological aqueous phase is on one side of the biofilm while the gas phase is on the other side. VOC contaminants and oxygen diffuse through the membrane, from the gas phase to the aqueous phase, where they are degraded. Currently, membrane bioreactors are also being investigated for treatment of

contaminated vapors (McGrath and Ergas, 1995; Reij et al., 1995). These systems operate as fixed-film systems, with biomass growing on the "tube" or "shell" side of a membrane-contacting device. As a vapor stream flows through either the "shell" or "tube" side of the system, mass transfer of the contaminants across the membrane boundary occurs, with the subsequent degradation of the contaminants in the biofilm. The biofilm is kept moistened by a flow of recirculating medium across the biofilm layer. The medium is also used for pH and other process control. A membrane system is currently being tested by Envirogen for TCE treatment. This technology is relatively new, and the range of applicability has not yet been determined.

7.0 BIOGAS REACTOR TECHNOLOGY

Specific process control parameters will influence the biological reactor process selection and its design. Those parameters include contaminant mass transfer requirements from the gas phase to the aqueous phase, cosubstrate feeding and process control requirements, pH and alkalinity control, target VOC DREs, and volumetric flow capacity. An additional consideration is the potential for anaerobic biological activity to promote CAC dechlorination in the reactors. The four reactor configurations being considered for the off-gas treatment process are conventional biofilters, fixed-film trickling filters, bioscrubbers, and complete-mix reactors with air sparging. Membrane bioreactors also offer attractive advantages for off-gas biological treatment, but they remain in their infancy technologically and would require significantly more development before being considered for such a complex off-gas stream.

The McClellan AFB off-gas contains a very complex mixture of contaminants, including some CACs that are relatively difficult to degrade. Many of these CACs are degraded cometabolically by the action of nonspecific oxygenase enzymes, and the CAC-degrading oxygenase enzymes are produced by bacteria when they are grown on a select group of cosubstrates such as methane, ammonia, propane, propylene, toluene, phenol, or IPB. One major contaminant, PCE, can be degraded only under anaerobic conditions via reductive dechlorination. The system configuration must be capable of (1) allowing for the effective addition of the cosubstrate and its efficient degradation within the biological reactor; (2) controlling process pH, alkalinity, temperature, and other physical/chemical parameters; and (3) economically processing the required vapor throughput. Another design consideration is the possible development of anaerobic zones within a biofilm to promote reductive dechlorination.

7.1 Contaminant Mass Transfer Requirements

The biological treatment of the SVE off-gas and the efficient removal of the off-gas contaminants from the gas phase require the efficient mass transfer of the VOCs to the aqueous phase of the reactor. Bioscrubbers rely on the mass transfer of the contaminants from the off-gas to the aqueous phase, followed by the biological treatment of the contaminated effluent water in a separate stage. The efficient use of an aqueous bioscrubber requires that the contaminants have relatively low Henry's coefficients of less than 10^{-3} atm-m^3/mol. Removal of compounds that have Henry's constants greater than 10^{-3} atm-m^3/mol is limited by mass transfer, resulting in low removal efficiencies ($\leq$ 50%) (Overcamp et al., 1993). Except for acetone and the ketones, most of the compounds present in the McClellan AFB off-gas stream are sparingly soluble in water and have Henry's constants greater than 10^{-3} atm-m3/mol. A bioscrubber would require in excess of 3,500 gpm of water to strip the contaminants from the off-gas, indicating that a bioscrubber would not be an effective system for the removal of the VOC contaminants from the off-gas. Thus, a bioscrubber system is not recommended for the off-gas treatment at McClellan AFB.

Conventional biofilters, complete-mix suspended-growth bioreactors, and biotrickling filters also rely on the efficient mass transfer of contaminants from the gas phase to the aqueous phase. Bioscrubbers rely on the separate removal of the contaminants into the aqueous phase and subsequent treatment of the contaminated water. In contrast, biofilters, complete-mix bioreactors, and biotrickling filters rely on concurrent biodegradation and contaminant mass transfer to the aqueous phase. In those three systems, the adsorption onto organic solids and biological activity contributes to the maintenance of very low aqueous phase VOC concentrations. The low aqueous-phase concentrations produce a large concentration gradient between the gas and aqueous phases, resulting in more efficient mass transfer.

TCE degradation has been demonstrated using complete-mix suspended-growth bioreactors (Ensley, 1992) and biotrickling filters (Govind et al., 1993; Govind et al., 1995) with DREs greater than 90%. Therefore, both systems are considered further for the McClellan AFB application. Less successful demonstrations have been made using conventional biofilters (see Section 6.1).

7.2 Requirements for Cosubstrate Feeding and CAC Degradation Rates

Because a significant fraction of the McClellan AFB SVE off-gas contaminants must be treated cometabolically, cosubstrate feeding is an important consideration in the system configuration. The low concentrations of toluene (and possibly other aromatic hydrocarbon compounds) already present in the

wastestream may contribute to a small fraction of the overall CAC cometabolic degradation, but the addition of another cosubstrate will be required for complete CAC degradation.

Both gaseous (methane, propane, and/or ammonia) and liquid (phenol, toluene, and/or IPB) cosubstrates can be readily added to the process water of complete-mix bioreactors and biotrickling filters.

The ability to control the cosubstrate feeding rate and the steady-state reactor cosubstrate concentration is vital to the efficient operation of the biological treatment process and the ability to achieve high DREs. The steady-state cosubstrate concentration within the reactor must be kept low to prevent competitive inhibition between the cosubstrate and the CACs, whereas sufficient cosubstrate must be added to the process to sustain an active biomass for cometabolic CAC degradation. Thus, careful process control of the cosubstrate fed to the reactor system is necessary. Steady-state cosubstrate concentrations must be controlled by influent flowrates and the liquid recycle rate. Liquid cosubstrates can be metered into the process water of biotrickling filters and suspended-growth bioreactors with relative ease; liquid cosubstrates cannot be added to biofilters easily, because biofilters are designed to operate with a stationary water phase and do not employ a liquid recycle system.

Introducing methane and propane into the process water is more difficult than adding liquid cosubstrates because methane and propane have high Henry's constants, and they do not readily partition from the gas phase into the aqueous phase. Thus, they cannot simply be metered into the influent gas stream because they will not partition into the aqueous phase quickly enough and will not be made available to the bacteria. Thus, a substantial fraction of the mass of cosubstrate added to the system will pass through the reactors unaltered.

A more effective method of adding gases to process water is to use a high-efficiency mass-transfer bubble contactor, similar to contactors used to supply pure oxygen to water in aerobic reactor systems (Sutton and Mishra, 1994). Oxygen bubble contactors are an integral part of Envirogen's fluidized-bed reactor (FBR) systems and can be modified for methane or propane addition to a bioreactor for this application.

As mentioned earlier, low cosubstrate concentrations must be maintained to minimize competitive inhibition effects on CAC degradation. Complete-mix bioreactors provide a well-mixed system that results in rapid dilution of influent substrates and low effluent concentrations, but they require relatively rapid substrate degradation rates; complete-mix reactors are less effective for compounds that exhibit first-order degradation characteristics, such as TCE (and presumably other CACs). Plug-flow reactors, on the other hand, are much more effective for compounds with first-order degradation rates. Biotrickling filters offer a plug-flow configuration. One disadvantage of plug-flow

reactors is that the cosubstrate concentrations may be too high at the beginning of the reactor, in spite of the fact that the effluent concentration is very low. The high influent concentrations could contribute to competitive inhibition of CAC degradation rates. By increasing the liquid recycle rate, the plug-flow reactor can be operated to act more like a complete-mix reactor, overcoming the problem of high influent cosubstrate concentrations, whereas the gas phase can be operated without recycle to maintain plug-flow operation of the gas phase. Thus, in spite of the fact that both biotrickling filters and complete-mix reactors can be operated with efficient contaminant mass transfer into the aqueous phase, biotrickling filters are expected to outperform complete-mix suspended-growth bioreactors because of the slow, first-order CAC degradation rates.

7.3 Alkalinity Requirements and pH Control

Conventional compost-based biofilters typically are not used for treatment of chlorinated compounds due to their inability to effectively control pH. The pH within conventional biofilters can be controlled to a limited extent by the addition of solid calcium carbonate or lime to the biofilter packing media at the beginning of operation. Once the buffering capacity is exhausted, the packing must be replaced. The amount of solid buffering material used depends on the application, but Ottengraf (1987) has specified a range of 2 to 40% (dry wt.) for most applications. Because of the high organic chlorine content in the SVE off-gas, if an empty bed vapor contact time of 2 min is assumed for a biofilter with 40% calcium carbonate ($CaCO_3$), the packing material will have to be replaced every 1.5 months. Because of the frequent bed replacement, biofilters are not recommended for this application.

Biotrickling filters and suspended-growth reactors are capable of effectively controlling pH by adding alkalinity (bicarbonate or carbonate) to the process water or by titrating NaOH into the process water using an electronic feedback system that monitors pH. The NaOH titration involves more risk than the buffering system, but is more economical for biological processes treating wastes with high organic chlorine concentrations and significant acid production.

7.4 Flow Capacity

Envirogen has modeled TCE degradation within its stirred-tank configuration using phenol as the cosubstrate and has shown that TCE removal is not limited by mass transfer, but is primarily limited by the biological degradation rate. At a volatile suspended solids (VSS) concentration of 5,000 mg/L and 90% TCE removal, the maximum volume of air that that can be processed through a complete-mix

bioreactor is 0.7 to 0.14 volume of contaminated air per min per volume of liquid suspension (vol/vol-min), depending on the inlet gas-phase TCE concentration, up to 500 ppmv (Envirogen, 1996; Radian/Envirogen, 1996). These flowrates correspond to an effective vapor contact time of 7 to 14 min (i.e., reactor volume divided by the vapor flowrate). For 95% TCE removal, the effective vapor contact time increases to 50 min (Envirogen, 1996). Thus, a 600-scfm reactor would require approximately 4,300 to 8,500 ft^3 to achieve 90% CAC removal, and up to 7 times those volumes to achieve 95% removal.

In contrast to the volume required for the complete-mix system, Govind et al. (1993) demonstrated 95% TCE removal using a biotrickling filter with an inlet TCE concentration of 25 ppmv. The reactor was operated with a 2- to 4-min vapor contact time (Govind et al., 1993). The lower contact times demonstrated for the biotrickling filter are attributed to the first-order TCE degradation kinetics; as mentioned above, a plug-flow reactor is expected to outperform a complete-mix reactor when reaction rates are first order, given similar vapor contact times (Andrews and Noah, 1995). In addition, because biotrickling filters employ a fixed-film process, the effective biomass concentration per unit volume of reactor can be maintained much higher than for a suspended-growth reactor (Andrews and Noah, 1995), resulting in higher performance per unit reactor volume.

7.5 Capacity for Anaerobic Treatment Zones

As discussed in Section 3, some of the compounds in the SVE off-gas stream can be dechlorinated biologically under anaerobic conditions. Two compounds, PCE and Freon™ 113, are aerobically recalcitrant and can be biotransformed only anaerobically. In spite of the fact that the biological processes will be operated aerobically, there is a strong possibility that anaerobic regions will be formed in a biological trickling filter process, whereas no such regions are possible in a complete-mix system. In the deeper layers of a biofilm, anaerobic areas where sulfate-reducing bacteria and methanogens can survive can be formed due to oxygen mass-transfer limitations in the biofilm (Arvin and Harremoes, 1990; Baltzis and Shareefdeen, 1994; Enzien et al., 1994). Enzien et al. (1994) reported the biodegradation of TCE and PCE under aerobic conditions in a sediment column; *c*-DCE was the major product of TCE and PCE dechlorination, no VC was detected, and the bulk liquid DO concentration was greater than 1.6 mg/L. In spite of the bulk aerobic conditions, methane was detected in the pore waters. The authors attributed the dechlorination to the development of anaerobic conditions in "microsites" within the soil matrix. To date, anaerobic sites within biofilms have not been exploited to promote reductive dechlorination of CACs under bulk aerobic conditions. Anaerobic pockets will not

be a controlling process selection and design factor for the off-gas biological treatment system, but the bioreactor design may include the flexibility of enhancing anaerobic activity via process control during the laboratory-phase study.

8.0 TECHNOLOGY SELECTION

Based on the discussion in Section 7, the biotrickling filter is the most suitable reactor configuration for the McClellan AFB SVE off-gas application. Biotrickling filters (1) can be operated with high contaminant mass-transfer rates, (2) allow for the efficient addition and control of liquid- or gas-phase cosubstrates, (3) provide a plug-flow configuration for efficient degradation of contaminants that exhibit first-order degradation rates, (4) permit relatively simple pH control via alkalinity addition or NaOH titration, (5) economically process the required vapor throughput with low vapor-contact times, and (6) provide for the possibility of reductive dechlorination via the production of anaerobic pockets within the biofilm. Two parallel trickling biofilters will be operated in the laboratory. The two biotrickling filter units will be used to test different cosubstrates and different operating parameters to optimize vapor throughput, contaminant DREs, and cosubstrate use. In this section, the biotrickling filter design approach is presented in greater detail.

8.1 Cosubstrate Selection

Cosubstrate selection is an important design consideration for the off-gas wastestream. The cosubstrate-degrading bacteria must be able to degrade chloroethenes and chloroethanes, they must be able to degrade CACs and the cosubstrate simultaneously, and they must be resistant to CAC intermediate toxicity. Among the cosubstrates reported in the literature including methane, propane, propylene, ammonia, toluene, phenol, and IPB, only methanotrophs, propane-oxidizers, ammonia-oxidizers, and IPB-oxidizers have been shown to degrade chloroethenes and chloroethanes. Ammonia is not suitable due to severe competitive inhibition expressed by ammonia on TCE degradation rates. Methane is unsuitable because methanotrophic cultures are relatively susceptible to intermediate product toxicity, exhaustion of intracellular energy (i.e., NADH), and inhibition of sMMO production in the presence of high copper concentrations.

IPB and propane remain as potential cosubstrates. The fact that limited information is available on both cosubstrates is a concern, but does not outweigh the fact that both cosubstrates support

chloroethene and chloroethane degradation, and both have been demonstrated to resist intermediate toxicity. IPB has the added advantage of being soluble, simplifying its addition to the liquid stream.

Because of the importance of the cosubstrate and the development of a mixed biological culture capable of efficient CAC degradation, flexibility must be incorporated into the system design to be able to change cosubstrates during the study should either propane or IPB fail to meet the DRE requirements. In addition to the cosubstrate selection, the cosubstrate/contaminant feeding ratio (R_S) is also an important design consideration. This ratio is the inverse of the transformation yield (T_Y), used by Dolan and McCarty (1995). Using propane as a cosubstrate, Phelps et al. (1990) reported a value of 26 to 34 g of propane consumed per g of TCE degraded, whereas Chang and Alvarez-Cohen (1995) reported a value of 43 g of propane per g of TCE degraded.

R_S values for IPB have not been reported in the literature, but their values are expected to be similar to those of phenol and toluene, because all three are aromatic compounds and are structurally similar. For toluene, Chang and Alvarez-Cohen (1995) reported a value of 21 g of toluene consumed per g TCE degraded for a mixed toluene-degrading culture; Ensley (1992) reported an average value of 22 g of toluene per g TCE degraded for *P. mendocina* in a bubble-column reactor; Govind et al. (1993) reported 15 g of toluene per g of TCE in biotrickling filters. For phenol, Hopkins et al. (1993) reported a value of 16 g of phenol per g of TCE during a field-pilot test, and 9 g of phenol per g TCE degraded for resting cells in the laboratory. Ensley (1992) reported an average value of 6 g of phenol per g TCE degraded for *P. cepacia* G4 in a bubble column reactor and saw TCE degradation at phenol concentrations as low as 2 g phenol per g TCE.

The values described above do not necessarily represent optimal R_S values, because not all studies attempted to optimize the cosubstrate-to-TCE ratio. The initial cosubstrate to CAC ratios for propane and IPB during the laboratory-phase tests will be based on the values reported above for propane and phenol (i.e., 30 g propane per g CAC and 6 g IPB per g CAC). The low R_S value for IPB is based on Envirogen's experience using phenol as the cosubstrate for TCE degradation within stirred-tank reactors. The more conservative value for propane will be used because there is limited background literature that describes the use of propane as a cosubstrate for cometabolism of CACs in biological reactors. In addition, the excess propane will be needed to maintain adequate propane concentrations in the aqueous phase of the reactor. Because of the scale of the laboratory reactors, a high-efficiency mass transfer device will not be employed for propane during the laboratory phase, and propane will be added in the gas phase. This will result in less efficient propane utilization, but will be less expensive and easier to control in the laboratory.

Aqueous-phase propane concentrations will be controlled by regulating the partial pressure of propane in the gas phase. Aqueous-phase IPB concentrations will be controlled by regulating the IPB feed rate via the feed concentration, and by regulating the liquid recycle rate.

8.2 System Configuration

Selective pressures in a complex, mixed biological community will result in the growth and proliferation of bacteria that receive the greatest benefit from the available substrate(s), followed by the growth of bacteria that gain progressively less energy for cell growth and maintenance. In the absence of a cosubstrate, the NCOCs, including acetone, BTEX, and ethyl toluene, will be degraded first by bacteria that that can beneficially exploit energy and carbon for growth from those compounds. Bacteria that degrade MEK or MIBK will grow next, followed by those that degrade DCBs. This kind of stratification is based on the available energy from each group of compounds and the relative ease for the bacteria to exploit that energy.

Addition of the cosubstrate in the presence of the NCOCs and DCBs in the off-gas may result in biological competition between those compounds in the biotrickling-filter reactor. For example, if IPB is added before the acetone is degraded, bacteria that can degrade either IPB or acetone will most likely preferentially degrade the acetone, followed by IPB. On the other hand, those that degrade both IPB and DCBs may be expected to preferentially degrade IPB, leaving the DCBs undegraded until the IPB is exhausted from the liquid stream. Thus, the addition of the cosubstrate in the presence of other growth-related substrates may result in competition between those substrates and in the inefficient use of the cosubstrate. Because CACs do not provide carbon and energy for cell growth, their degradation is not expected to confer any selective advantage to the CAC-degrading bacteria. Therefore, the cosubstrate should be added to the biotrickling filter column after the gas-phase growth substrates (acetone, BTEX, ethyl toluene, MEK, MIBK, and DCBs) have been consumed. This will be accomplished by staging the biotrickling filter. Two-stage columns will be used. The first stage will not be fed a cosubstrate and will be used to promote the degradation of growth-related contaminants in the off-gas. The removal of those contaminants will result in a simplified gas stream that will be fed to the second stage. The second stage will be used to promote cometabolic CAC degradation, using either propane or IPB as cosubstrates. Figure 3 shows a schematic of the proposed system configuration. Independent liquid feed and recycle streams will be maintained for each reactor stage. Alkalinity and pH also will be controlled independently for each stage. The gas will be fed to the bioreactors cocurrent with the direction of the liquid flow, which will be fed to the top of each reactor and allowed to trickle down via gravity flow.

The off-gas from the first stage will be fed to the second stage. At a minimum, the reactors will be monitored at the influent and effluent of both reactor stages.

8.3 Vapor Throughput

The vapor throughput of the biotrickling filter systems will be dictated by the vapor contact time required for contaminant removal to predetermined DREs (i.e., 95%). The vapor contact time is defined as the reactor volume occupied by packing divided by the vapor flowrate. The reactor dimensions (i.e., the height-to-diameter ratio) are dictated by the maximum allowable pressure drop and head loss for the reactor system. For TCE degradation, Govind et al. (1993) demonstrated 95% TCE removal in a biotrickling filter system containing activated carbon as the packing material and using an empty bed vapor contact time of 2 to 4 min. Because carbon, which can provide buffering capacity for shock loads, will not be used for this application, and because of the complexity of the wastestream, a longer vapor contact time of 4 to 5 min will be employed during the laboratory-phase test.

8.4 Liquid (and Biomass) Discharge

Liquid must be discharged from the biotrickling filter system, either continuously or intermittently, to prevent the accumulation of sodium chloride (NaCl) and the exhaustion of inorganic nutrients supplied in the process water. NaCl will be produced from chloride ions released during CAC degradation. The NaCl concentration will be maintained below 2%. However, NaCl concentrations in biofilters have been reported to reach as high as 2.2 to 3.5% (Diks et al., 1994a) and 2.5% (Envirogen, unpublished results) without adversely affecting the contaminant degradation characteristics. The liquid discharge also will contain low biomass concentrations. The biomass concentration will depend on the cosubstrate mass fed to the reactors and the biomass yield of the cosubstrate.

Continuous liquid wasting also will prevent the accumulation of process water fed to the system. The system waste rate will be adjusted to prevent NaCl accumulation, to maintain low aqueous-phase cosubstrate concentrations, to allow sufficient cosubstrate and inorganic nutrient feeding, and to control biomass sloughing from the biofilm.

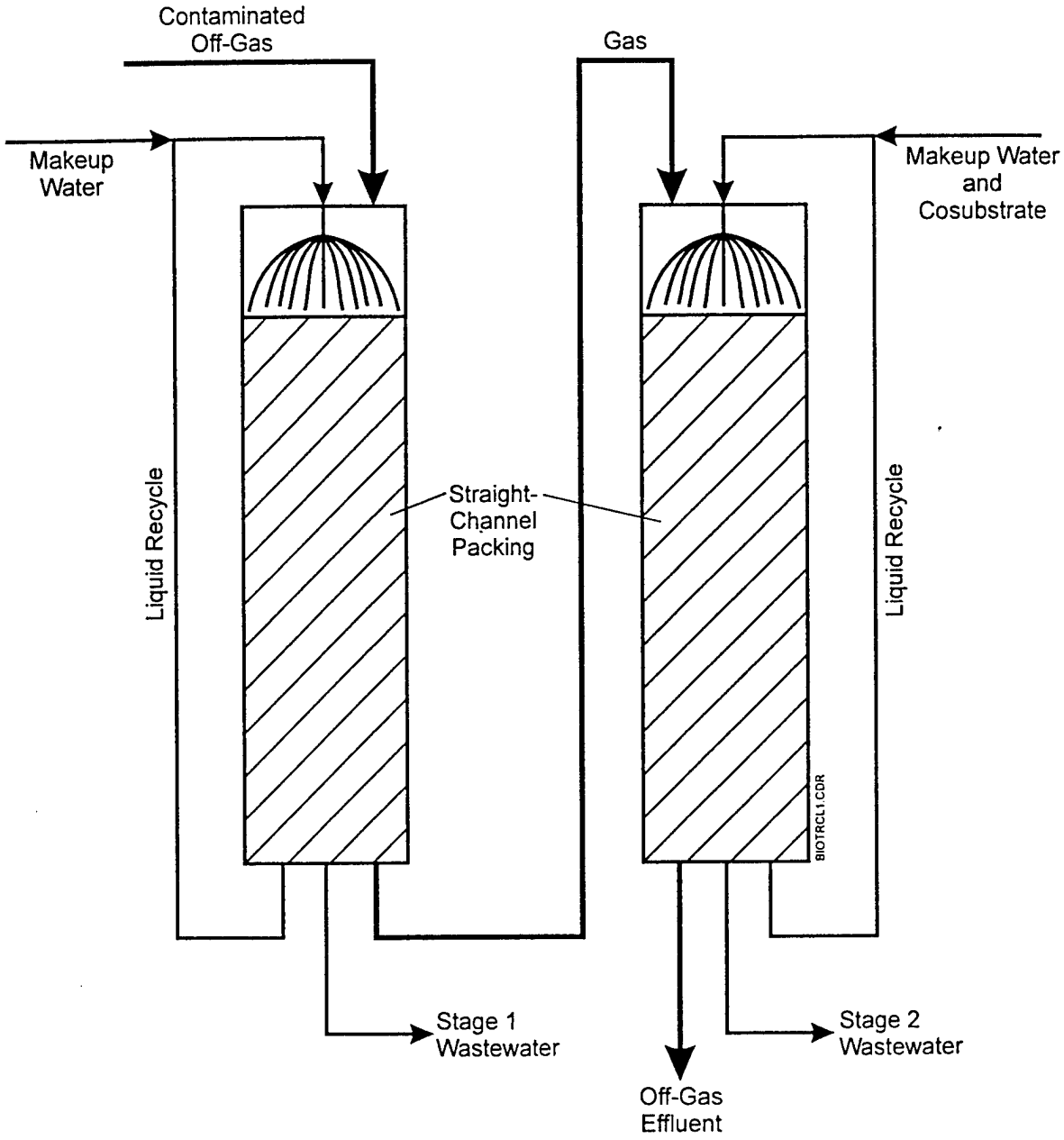

Figure 3. Biotrickling Filter Reactor System Configuration

8.5 Upsets Due to Shock Loads

The use of powdered activated carbon (PAC) in the recirculating water of bioscrubbers has recently been proposed as a way of enhancing the solubility and degradability of sparingly soluble contaminants (Overcamp et al., 1994; Kok, 1994). PAC also can protect the biological system against organic shock loads. The bench-scale systems will be operated at steady-state feed conditions, so PAC will not be required. However, if time permits, PAC will be added to the recirculating water to investigate the effects of PAC on VOC control and removal from the off-gas. The risk of shock loads is much greater in the field and during full-scale operation than in the laboratory.

Another method of controlling shock loads is to monitor the influent gas stream and to control the gas flow based on the VOC concentrations in the off-gas to maintain a consistent mass flowrate to the biological treatment system. This method will require accurate process control of the SVE off-gas systems at the site.

8.6 Reactor Plugging

Envirogen's biotrickling filter systems are designed to prevent biomass plugging and excessive packing pressure drops. Typically, the pressure drop across the packing is no more than 5 or 6 in. of water column. This is accomplished by operating the systems in a downflow configuration at appropriate liquid and vapor velocities. Biomass growth is controlled by the liquid and vapor shear forces. Envirogen has developed a family of pressure-drop curves for different packing materials under conditions of actively growing biofilm on the packing; these pressure-drop curves are unique to Envirogen's reactors and packing materials and have been developed for field-pilot and full-scale systems. Because of scale-up limitations, the bench-scale systems will not be operated to control pressure drop. However, the pressure drop across the packing will be monitored in the laboratory to provide information that will contribute to the scale-up to pilot- and full-scale systems, along with Envirogen's pressure drop curves.

For the McClellan AFB application, biomass growth also may be controlled by the relatively high NaCl concentrations (Diks et al., 1994a). The actual benefit of the high NaCl concentrations for the control of the reactor biomass processes cannot be determined at this time, but NaCl concentrations will be monitored closely and adjusted for maximum control.

8.7 System Optimization

The system will be optimized to maximize vapor throughput, maximize individual VOC DREs, minimize cosubstrate use, and promote anaerobic activity within the biofilm. Vapor throughput will be maximized to determine the minimum vapor contact time to achieve the desired DREs. Vapor throughput and VOC DREs will be interdependent, and changing one will most likely change the other. DREs will be maximized by controlling vapor throughput for the two stages, controlling cosubstrate addition, and maintaining optimal pH and NaCl concentrations. Cosubstrate use will be optimized by determining the minimum cosubstrate loading rate while maintaining predetermined DREs.

Anaerobic zones in the biofilm may be exploited for the degradation of PCE, TCE, 1,1,1-TCA, and/or Freon™ 113. The anaerobic zones may be created in the deeper layers of the biofilm in the biotrickling filters, creating areas where anaerobic bacteria can survive and proliferate (Arvin and Harremoes, 1990; Enzien et al., 1994). Reductive dehalogenation may occur in either the first or second stage. The first stage will be supplied with easily degraded organic substrates in the gas phase as potential electron donors for reductive dehalogenation, and the second stage will be supplied with an organic cosubstrate that also may act as an electron donor. In the absence of anaerobic activity during normal operation, an easily degraded growth substrate, such as lactate, may be added to the recirculating water in either stage to promote a thicker biofilm and anaerobic activities within the biofilm.

8.8 Summary of Literature Review and Technology Recommendation

Based on the characteristics of the off-gas stream and a review of available biological treatment options, the biotrickling filter process configuration was selected as the best process for the McClellan AFB SVE off-gas stream. Conventional compost-based biofilters would require frequent bed replacement, and the high Henry's law constants of most of the contaminants in the off-gas prevent the economical use of a bioscrubber system. Slow, first-order degradation rates of the CACs in the off-gas prevented the selection of a complete-mix suspended-growth biological reactor.

Because of the complexity of the McClellan AFB off-gas stream, and because of presence of growth-supporting contaminants with contaminants that do not support growth, a two-stage biotrickling filter system is proposed. The first stage will be used to remove the easily degraded growth substrates from the stream, such as acetone, BTEX, MEK, MIBK, and DCBs. The second stage will be used to cometabolically degrade CACs in the off-gas. Two cosubstrates will be investigated initially, using two separate two-stage reactor units. Propane and IPB were selected as the cosubstrates for this

investigation, because of their abilities to support bacteria that can degrade chloroethenes and chloroethanes, and because of their potential resistance to intermediate toxicity and competitive inhibition with CACs. Propane will be added to the influent gas stream, and IPB will be metered into the liquid recirculation line.

The two separate reactor units will provide greater opportunity to optimize the system with respect to vapor throughput, contaminant DREs, cosubstrate use, and possibly anaerobic activity to promote reductive dechlorination in the anaerobic zones within the biofilm. As a starting point for bench-scale optimization, a vapor contact time of 4 to 5 min will be employed. Initially, a propane-to-CAC ratio of 30 will be used, and an IPB to CAC ratio of 6 will be used. Aqueous-phase propane concentrations will be controlled by adjusting the partial pressure of propane in the gas phase. Aqueous-phase IPB concentrations will be controlled by controlling the influent IPB loading rate and the liquid recirculation rate. The contaminated off-gas stream will be fed into the system with a single pass; the gas phase will not be recirculated in the system.

The liquid removal rates from each stage will be set initially to maintain an NaCl concentration below 2.0%. Optimization may include the use of PAC in the recirculation water in the second stage of both systems to investigate the effects of PAC on system performance. PAC may enhance the solubility and degradability of sparingly soluble contaminants and can protect the system against organic shock loads. It also can provide a method of regulating the supply of cosubstrate to the reactors by having a cosubstrate/PAC contact unit. Another unique treatment approach is the use of high-substrate feed zones to stimulate anaerobic activity in the biofilm for anaerobic reductive CAC dechlorination.

9.0 REFERENCES

Allen, E.R., and Y. Yang. 1992. "Biofiltration: An air pollution control technology for hydrogen sulfide emissions," in *Industrial Environmental Chemistry*, D.T. Sawyer and A.E. Martell, Eds., Plenum Press, New York):273-287.

Alvarez-Cohen, L., and P.L. McCarty. 1991a. "Effects of Toxicity, Aeration, and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture." *Appl. Environ. Microbiol. 57*(1):228-235.

Alvarez-Cohen, L., and P.L. McCarty. 1991b. "Two-Stage Dispersed Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism." *Environ. Sci. Technol. 25*:1387-1393.

Anderson, W.C. 1994. Innovative Site Remediation Technology. American Academy of Environmental Engineers. Annapolis, MD. pp. 116.

Andrews, G.F., and K.S. Noah. 1995. "Design of gas-treatment bioreactors." *Biotechnol. Prog. 11*(5):498-509.

Apel, W.A., W.D. Kant, F.S. Colwell, B. Singleton, B.D. Lee, G.F. Andrews, A.M. Espinosa, and E.G. Johnson. 1993. Removal of Gasoline Vapors from Air Streams by Biofiltration. Report No. EGG-WTD-10714, Idaho National Engineering Laboratory, EG&G Idaho, Inc.

Arciero, D., T. Vannelli, M. Logan, and A.B. Hooper. 1989. "Degradation of Trichloroethylene by the Ammonia-Oxidizing Bacterium *Nitrosomonas Europaea*." *Biochemical and Biophysical Research Communications. 159*(2):640-643.

Arvin, E., and P. Harremoes. 1990. "Concepts and models for biofilm reactor performance." *Wat. Sci. Tech. 22*(1-2):171-192.

Baltzis, B.C., and Z. Shareefdeen. 1994. "Biofiltration of VOC Mixtures: Modeling and Pilot Scale Experimental Verification," paper no. 94-TA260.10P, 87th Annual Air & Waste Management Association Meeting & Exhibition, Cincinnati, OH, June 19-24.

Begley, R. 1991. "Industry watches closely as EPA converts the clean air act into regulation." *Chemical Week.* 13:23-28.

Bielefeldt, A.R., H.D. Stensel, and S.E. Strand. 1995. "Cometabolic Degradation of TCE and DCE Without Intermediate Toxicity." *J. of Environ. Engin. 121*:791-797.

Bohn, H. 1992. "Consider biofiltration for decontaminating gases." *Chem. Eng. Prog.* 88(4):34-40.

Bohn, H., and R. Bohn. 1988. "Soil beds weed out air pollutants." *Chemical Engineering.* 95(4):74-76.

Broholm, K., T.H. Christensen, and B.K. Jensen. 1993. "Different Abilities of Eight Mixed Cultures of Methane-Oxidizing Bacteria to Degrade TCE." *Wat. Res.* 27(2):215-224.

Capel, P., Leunberger, and Giger. 1991. "Hydrophobic organic chemicals in urban fog." *Atmospheric Environ.* 25A(7):1335-1346.

Castro, C.E., and N.O. Belser. 1990. "Biodehalogenation: Oxidative and Reductive Metabolism of 1,1,2,-Trichloroethane by *Pseudomonas Putida*) Biogeneration of Vinyl Chloride." *Environmental Toxicology and Chemistry. 9*:707-714.

Chapman, T (BDM). 1996. Personal communication with V. Magar (Battelle).

Chang, H-L., and L. Alvarez-Cohen. 1995. "Model for the cometabolic biodegradation of chlorinated organics." *Environ. Sci. Technol.* 29:2357-2367.

Chen, C., J.A. Puhakka, and J.F. Ferguson. 1996. "Transformations of 1,1,2,2-Tetrachloroethane under Methanogenic Conditions." *Environ. Sci. Technol.* 30:542-547.

Chetty, A.S., J.A. Dyer, and K.L. Mulholland. 1992. "The Role of Economics in Selecting VOC Controls," paper no. 92-114.13, 85th Annual Air & Waste Management Association Meeting & Exhibition, Kansas City, MO, June 21-26.

Croonenberghs, J., F. Varani, and P. Le Fevre. 1994. "Use of Bioscrubbing to Control Ethanol Emissions," paper no. 94-RP115B.06, 87th Annual Air & Waste Management Association Meeting & Exhibition, Cincinnati, OH, June 19-24, 1994.

Dabrock, B., J. Riedel, J. Bertram, and G. Gottschalk. 1992. "IPB (cumene)—A New Substrate for the Isolation of Trichloroethene-Degrading Bacteria." *Arch Microbiol 158*:9-13.

Dasu, B.N., V. Deshmane, R. Shanmugasundram, C.M. Lee, and K.L. Sublette. 1993. "Microbial reduction of sulfur oxide and nitric oxide." *Fuel. 72*:1705-1714.

de Bont, J.A.M., M.J.A.W. Vorage, S. Hartmans, and W.J.J. van den Tweel. 1986. "Microbial degradation of 1,3-dichlorobenzene." *Appl. Environ. Microbiol. 52*:677-680.

Debus, O., H. Baumgärtl, and I. Sekoulov. 1994. "Influence of Fluid Velocities on the Degradation of Volatile Aromatic Compounds in Membrane Bound Biofilms." *Wat. Sci. Tech. 29*:253-262.

DeFilippi, L.J., M.B. Koch, C.M. Voellinger, D.R. Winstead, and F.S. Lupton. 1993. "A Biological Air Treatment System Based Upon the Use of a Structured Carbon Biomass Support." Presented at the IGT Symposium on Gas, Oil, and Environmental Biotechnology, Colorado Springs, CO, Nov. 29 - Dec. 1, 1993.

Devinny, J.S., and D.S. Hodge. 1995. "Formation of acidic and toxic intermediates in overloaded ethanol biofilters." *J. Air Waste Manage. Assoc.* 45(2):125-131.

Dharmavaram, S. 1991. "Biofiltration - A Lean Emissions Abatement Technology," paper no. 91-103.2, 84th Annual Air & Waste Management Association Meeting & Exhibition, Vancouver, British Columbia, June 16-21.

Dharmavaram, S., U.C. Duursma, G. Rietbroek, and E. Waalewijn. 1995. "Use of a Biotrickling Filter (BTF) for Control of N,N-Dimethylacetamide Emissions," paper no. 95-TA9B.01, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23.

Diks, R.M.M., and S.P.P. Ottengraf. 1991a. "Verification studies of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter (Part I)." *Bioprocess Engr.* 6:93-99.

Diks, R.M.M., and S.P.P. Ottengraf. 1991b. "Verification studies of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter (Part II)." *Bioprocess Engr.* 6:131-140.

Diks, R.M.M., S.P.P. Ottengraf, and A.H.C. van den Oever. 1994a. "The influence of NaCl on the degradation rate of dichloromethane by *Hyphomicrobium* sp." *Biodegradation.* 5:129-141.

Diks, R.M.M., S.P.P. Ottengraf, and S. Vrijland. 1994b. "The existence of a biological equilibrium in a trickling filter for waste gas purification." *Biotechnol. Bioeng.* 44:1279-1287.

Dolan, M.E. and P.L. McCarty. 1995. "Methanotrophic chloroethene transformation capacities and 1,1-dichlorethene transformation product toxicity." *Environ. Sci. Technol. 29*:2741-2747.

U.S. Department of Energy (DoE). 1994. Field Demonstration of Vapor Phase TCE Bioreactor. DOE/OR/21400-T492, prepared for the U.S. Department of Energy.

Dyer, J., and K. Mulholland. 1994. "Toxic air emissions: What is the full cost to your business?" *Environ. Engin. (supplement to Chemical Engineering). February*:4-8.

Ensign, S.A., M.R. Hyman, and D.J. Arp. 1992. "Cometabolic Degradation of Chlorinated Alkenes by Alkene Monooxygenase in a Propylene-Grown *Xanthobacter* Strain." *Appl. Environ. Microbiol. 58*(9):3038-3046.

Ensley, B.D. 1992. Biodegradation of Chlorinated Hydrocarbons in a Vapor Phase Reactor. DOE/CH-9207, prepared for the U.S. Department of Energy.

Ensley, B.D., and P.R. Kurisko. 1994. "A gas lift bioreactor for removal of contaminants from the vapor phase." *Appl. Environ. Microbiol.* 60(1):285-290.

Envirogen. 1996. Final report for DOD contract FO8635-91-C-0198 [Phase II], "Liquid-phase Bioreactor for Degradation of Trichloroethylene and Benzene." In press.

Enzien, M.V., F. Picardal, T.C. Hazen, R.G. Arnold, and C.B. Fliermans. 1994. "Reductive Dechlorination of Trichloroethylene and Tetrachloroethylene Under Aerobic Conditions in a Sediment Column." *Appl. Environ. Microbiol. 60*(6):2200-2204.

Ergas, S.J., E.D. Schroeder, and D.P.Y. Chang. 1992. "Biodegradation Technology for Volatile Organic Compound Removal from Airstreams - Phase I: Performance Verification." Final report for contract no. AO32-127. Prepared for the California EPA Air Resources Board, Research Division. May.

Ergas, S.J., E.D. Schroeder, and D.P.Y. Chang. 1993. "Control of Air Emissions of Dichloromethane, Trichloroethene, and Toluene by Biofiltration," paper no. 93-WA-52B.01, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18, 1993.

Ergas, S.J., K. Kinney, M.E. Fuller, and K.M. Scow. 1995. "Characterization of a compost biofiltration system degrading dichloromethane." *Biotechnol. Bioeng. 44*:1048-1054.

Fogel, M.M., A.R. Taddeo, and S. Fogel. 1986. "Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture." *Appl. Environ. Microbiol. 51*(4):720-724.

Folsom, B. 1992. "Liquid-phase Bioreactor for Degradation of Trichloroethylene and Benzene." DOD final report no. AD-D-275035/4/XAB.

Folsom, B.R., and P.J. Chapman. 1991. "Performance Characterization of a Model Bioreactor for the Biodegradation of Trichloroethylene by *Pseudomonas cepacia* G4." *Appl. Environ. Microbiol. 57*(6):1602-1608.

Folsom, B.R., P.J. Chapman, and P.H. Pritchard. 1990. "Phenol and Trichloroethylene Degradation by *Pseudomonas cepacia* G4: Kinetics and Interactions Between Substrates." *Appl. Environ. Microbiol. 56*(5):1279-1285.

Fouhy, K. 1992. "Cleaning waste gas, naturally." *Chemical Engineering. 99*(12):41-46.

Fox, B.G., J.G. Borneman, L.P. Wackett, and J.D. Lipscomb. 1990. "Haloalkene Oxidation by the Soluble Methane Monooxygenase from *Methylosinus trichosporium* OB3b: Mechanistic and Environmental Implications." *Biochemistry 29*:6419-6427.

Freedman, D.L., and S.D. Herz. 1996. "Use of Ethylene and Ethane as Primary Substrates for Aerobic Cometabolism of Vinyl Chloride." *Water Environ. Res. 68*(3):320-328.

Gälli, R., and P.L. McCarty. 1989. "Biotransformation of 1,1,1-Trichloroethane, Trichloromethane, and Tetrachloromethane by a *Clostridium* sp." *Appl. Environ. Microbiol. 55*(4):837-844.

Glotfeldy, D.E., M.S. Majewski, and J.M. Seiber. 1990. "Distribution of several organophosphorus insecticides and their oxygen analogues in a foggy atmosphere." *Environ. Sci. Technol. 24*(3):353-357.

Glotfelty, D.E., J.N. Seiber, and L.A. Lilijedahl. 1987. "Pesticides in fog." *Nature. 325*:602-605.

Govind, R., P.S.R.V. Prasad, and D.F. Bishop. 1995. "Anaerobic/Aerobic Degradation of Aliphatic Chlorinated Hydrocarbons in an Encapsulated Biomass Biofilter." Presented at the EPA Symposium on Bioremediation of Hazardous Wastes: Research, Development, and Field Evaluations, Rye Brook, NY, Aug. 8-10. Abstracts document no. EPA/600/R-95/076):50-52.

Govind, R., V. Utgikar, W. Zhao, Y. Shan, M. Parvatiyar, and D.F. Bishop. 1993. "Development of Novel Biofilters for Treatment of Volatile Organic Compounds (VOCs)." Presented at the IGT Symposium on Gas, Oil & Environmental Biotechnology, Colorado Springs, CO, Nov. 29 - Dec. 3.

Griffiths, C., J. Graydon, M. de Menibus, and G. Boyer. 1995. "Methylotrophic Biofiltration of Chlorinated Aliphatic Hydrocarbons." Presented at the 1995 Battelle In-situ and On-site Bioreclamation Conference in San Diego, CA.

Haigler, B.E., S.F. Nishino, and J.C. Spain. 1988. "Degradation of 1,2-dichlorobenene by a *Pseudomonas* sp." *Appl. Enviorn. Microbiol.* 54:294-301.

Harker, A.R., and Y. Kim. 1990. "Trichloroethylene Degradation by Two Independent Aromatic Degrading Pathways in *Alcaligenes eutrophus* JMP134." *Appl. Environ. Microbiol. 56*(4):1179-1181.

Hartmans, S., and J. Tramper. 1991. "Dichloromethane removal from waste gases with a trickle-bed bioreactor." *Bioprocess Eng. 6*:83-92.

Hartmans, S., and J.A.M. de Bont. 1992. "Aerobic Vinyl Chloride Metabolism in *Mycobacterium aurum* L1." *Appl. Environ. Microbiol. 58*(4):1220-1226.

HazTech News. 1996. "TVA Biofilter to be Tested (Cont.)." April 11:52.

Heald, S., and R.O. Jenkins. 1994. "Trichloroethylene Removal and Oxidation Toxicity Medicated by Toluene Dioxygenase of *Pseudomonas putida.*" *Appl. Environ. Microbiol. 60*(12):4634-4637.

Hecht, V., D. Brebbermann, P. Bremer, and W.D. Deckwer. 1995. "Cometabolic Degradation of Trichloroethylene in a Bubble Column Bioscrubber." *Biotechnol. Bioengin. 47*(4):461-469.

Heller, K. 1991. "Clean air: a fresh challenge 'preempt the rules with volunteerism." *Chemical Week.* Nov. *13*:22-23.

Henry, S.M., and D. Grbić-Galić. 1991. "Influence of Endogenous and Exogenous Electron Donors and Trichloroethylene Oxidation Toxicity on Trichloroethylene Oxidation by Methanotrophic Cultures from a Groundwater Aquifer." *Appl. Environ. Microbiol. 57*(1):236-244.

Hodge, D.S., and J.S. Devinny. 1994. "Biofilter treatment of ethanol vapors." *Environ. Prog. 13*(3):167-173.

Hodge, D.S., V.F. Medina, R.L. Islander, and J.S. Devinny. 1991. "Treatment of hydrocarbon fuel vapors in biofilters." *Environ. Technol. 12*:655-662.

Holubar, P., C. Andorfer, and R. Braun. 1995. "Prevention of Clogging in Trickling Filters for Purification of Hydrocarbon-Contaminated Waste Air," *Proceedings of the 1995 Conference on Biofiltration*, University of Southern California, October 5-6, pp. 115-121.

Hopkins, G.D., J. Munakata, L. Semprini, and P.L. McCarty. 1993a. "Trichloroethylene Concentration Effects on Pilot Field-Scale In-Situ Groundwater Bioremediation by Phenol-Oxidizing Microorganisms." *Environ. Sci. Technol. 27*:2542-2547.

Hopkins, G.D., L. Semprini, and P.L. McCarty. 1993b. "Microcosm and In Situ Field Studies of Enhanced Biotransformation of Trichloroethylene by Phenol-Utilizing Microorganisms." *Appl. Environ. Microbiol. 59*(7):2277-2285.

Hur, H.G., M.J. Sadowsky, and L.P. Wackett. 1994. "Metabolism of Chlorofluorocarbons and Polybrominated Compounds by *Pseudomonas putida* G786(pHG-2) via an Engineered Metabolic Pathway." *Appl. Environ. Microbiol. 60*(11):4148-4154.

Hyman, M.R., S.A. Russell, R.L. Ely, K.J. Williamson, and D.J. Arp. 1995. "Inhibition, Inactivation, and Recovery of Ammonia-Oxidizing Activity in Cometabolism of Trichloroethylene by *Nitrosomonas europaea." Appl. Environ. Microbiol. 61*(4):1480-1487.

Janssen, D.B., A. Scheper, L. Dijkhuizen, and B. Witholt. 1985. "Degradation of Halogenated Aliphatic Compounds by *Xanthobacter authotrophicus* GJ10." *Appl. Environ. Microbiol. 49*(3):673-677.

Ju, L.-K., and G.G. Chase. 1992. "Improved Scale-Up Strategies of Bioreactors." *Bioprocess Engineering 8*:49-53.

Kampbell, D.H., J.T. Wilson, H.W. Read, and T.T. Stocksdale. 1987. "Removal of volatile aliphatic hydrocarbons in a soil bioreactor." *JAPCA. 37*(10):1236-1240.

Kok, H.J.G. 1994. "Bioscrubbing of air contaminated with high concentrations of hydrocarbons." *Proceedings of the International Symposium on Biological Waste Gas Cleaning*, Heidelberg, Germany, March 9-11. VDI Berichte No. 1104.

Kuter, G.A., J.E. Harper, L.M. Naylor, and P.J. Gormsen. 1993. "Design, Construction and Operation of Biofilters for Controlling Odors at Composting Facilities," paper no. 93-WP-52C.02, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18.

Lacky, L., and T. Holt. 1996. "Not for the birds. Biofiltration using composted chicken litter efficiently removes styrene from industrial exhaust gases." *Industrial Wastewater.* May/June 1996:31-33.

Lam, T., and V.L. Vilker. 1987. "Biodehalogenation of Bromotrichloromethane and 1,2-Dibromo-3-chloropropane by *Pseudomonas putida* PpG-786." *Biotechnology and Bioengineering.* XXIX:151-159.

Landa, A.S., E.M. Sipkema, J. Weijma, A.A.C.M. Beenackers, J. Dolfing, and D.B. Janssen. 1994. "Cometabolic Degradation of Trichloroethylene by *Pseudomonas cepacia* G4 in a Chemostat With Toluene as the Primary Substrate." *Appl. Environ. Microbiol.* 60(9):3368-3374.

Langseth, S., and D. Pflum. March 1994. "Weyerhaeuser tests large pilot biofilters for VOCs removal." *Panel World.*

Lanzarone, N.A., and P.L. McCarty. 1990. "Column Studies on Methanotrophic Degradation of Trichloroethene and 1,2-Dichloroethane." *Ground Wat. 28*:810-191.

Leatherbarrow, R.J. 1990. "Use of Nonlinear Regression to Aanalyze Enzyme Kinetic Data: Application to Situations of Substrate Contamination and Background Subtraction." *Anal. Biochem. 184*:274-278.

Lee, J.Y, K.H. Jung, S.H. Choi, and H.S. Kim. 1995. "Combination of the *tod* and the *tol* Pathways in Redesigning a Metabolic Route of *Pseudomonas putida* for the Mineralization of a Benzene, Toluene, and *p*-Xylene Mixture." *Appl. Environ. Microbiol. 61*(6):2211-2217.

Lefever, M.R., and L.P. Wackett. 1994. "Oxidation of Low Molecular Weight Chloroalkanes by Cytochrome $P450_{CAM}$." *Biochem. and Biophysical Research Communications 201*(1):373-378.

Leson, G., and A.M. Winer. 1991. "Biofiltration: an innovative air pollution control technology for VOC emissions." *J. Air Waste Manage. Assoc. 41*(8):1045-1054.

Leson, G., and B.J. Smith. 1995. "Results from the PERF Field Study on Biofilters for Removal of Volatile Petroleum Hydrocarbons," *Proceedings of the 1995 Conference on Biofiltration*, University of Southern California, October 5-6:99-113.

Leson, G., D.S. Hodge, F. Tabatabai, and A.M. Winer. 1993. "Biofilter Demonstration Projects for the Control of Ethanol Emissions," paper no. 93-WP-52C.04, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18.

Li, S., and L.P. Wackett. 1993. "Reductive Dehalogenation by Cytochrome $P450_{CAM}$: Substrate Binding and Catalysis." *Biochemistry. 32:*9355-9361.

Little, C.D., A.V. Palumbo, S.E. Herbes, M.E. Lidstrom, R.L. Tyndall, and P.J. Gilmer. 1988. "Trichloroethylene Biodegration by a Methane-Oxidizing Bacterium." *Appl. Environ. Microbiol. 54*(4):951-956.

Loy, J. 1995. "Biological Elimination of Odoriferous Pollutants and Solvents in Waste Gas with the Biotrickling Filter," paper no. 95-MP9A.06, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23.

Mackay, D., and W.Y.Shiu. 1981. "Critical review of Henry's law constants for chemicals of environmental interest." *J. Phys. Ref. Data. 10*(4):1175-1199.

Malachowsky, K.J., T.J. Phelps, A.B. Teboli, D.E. Minnikin, and D.C. White. 1994. "Aerobic Mineralization of Trichloroethylene, Vinyl Chloride, and Aromatic Compounds by *Rhodococcus Species." Appl. Environ. Microbiol. 60*(2):542-548.

McFarland, M.J., C.M. Vogel, and J.C. Spain. 1992. "Methanotrophic Cometabolism of Trichloroethylene (TCE) in a Two Stage Bioreactor System." *Wat. Res. 26*(2):259-265.

McGrath, M.S., and S.J. Ergas. 1995. "Hollow Fiber Membrane Bioreactor for Control of Volatile Organic Compound Emissions," paper no. 95-TP9C.03, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23.

Mu, D.Y., and K.M. Scow. 1994. "Effect of Trichloroethylene (TCE) and Toluene Concentrations on TCE and Toluene Biodegradation and the Population Density of TCE and Toluene Degraders in Soil." *Appl. Environ. Microbiol. 60*(7):2661-2665.

Nelson, M.J.K., S.O. Montgomery, and P.H. Pritchard. 1988. "Trichloroethylene Metabolism by Microorganisms That Degrade Aromatic Compounds." *Appl. Environ. Microbiol. 54*(2):604-606.

Nelson, M.J.K.., S.O. Montgomery, E.J. O'Neill, and P.H. Pritchard. 1986. "Aerobic Metabolism of Trichloroethylene by a Bacterial Isolate." *Appl. Environ. Microbiol. 58*(2):383-384.

Nishino, S.F., J.C. Spain, L.A. Belcher, and C.D. Litchfield. 1992. "Chlorobenzene Degradation by Bacteria Isolated From Contaminated Groundwater." *Appl. Environ. Microbiol. 58*(5):1719-1726.

Ockeloen, H.F., T.J. Overcamp, and C.P.L. Grady, Jr. 1992. "A Biological Fixed-Film Simulation Model for Removal of Volatile Organic Pollutants," paper no. 92-116.05, 85th Annual Air & Waste Management Association Meeting & Exhibition, Kansas City, MO, June 21-26.

Oldenhuis, R. 1992. "Microbial Degradation of Chlorinated Compounds: Application of Specialize Bacteria in the Treatment of Contaminated Soil and Waste Water." PhD. Thesis. Rijksuniversiteit Groningen, Groningen, The Netherlands.

Oldenhuis, R., J.Y. Oedzes, J.J. van der Waarde, and D.B. Janssen. 1991. "Kinetics of Chlorinated Hydrocarbon Degradation by *Methylosinus trichosporium* OB3b and Toxicity of Trichloroethylene." *Appl. Environ. Microbiol. 57*:7-14.

Oldenhuis, R., R.L.J.M. Vink, D.B. Janssen, and B. Witholt. 1989. "Degradation of Chlorinated Aliphatic Hydrocarbons by *Methylosinus trichosporium* OB3b Expressing Soluble Methane Monooxygenase." *Appl. Environ. Microbiol. 55*(11):2819-2826.

Ottengraf, S.S.P. 1987. "Method for Biological Treatment of Waste Gases." U.S. patent no. 4,662,900.

Overcamp, T.J., B.F. Smets, R.E. Hammervold, and C.P.L. Grady, Jr. 1994. "A Bioregenerated Sorptive Slurry Scrubber," *in 49th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Chelsea, MI:419-424.

Overcamp, T.J., H-C Chang, and C.P.L. Grady, Jr. 1993. "An integrated theory for suspended growth bioscrubbers." *J. Air Waste Manage. Assoc. 43*(5):753-759.

Overcamp, T.L., H.F. Ockeloen, H-C Chang, and C.P.L. Grady, Jr. 1992. "Design Criteria for Bioscrubbers: Fixed-film Versus Suspended-growth Reactors." Presented at the 9th World Clean Air Conference and Exhibition, Montreal, Quebec, Canada, Aug. 30 - Sept. 4.

Pedersen, A.R., and E. Arvin. 1995. "Removal of Toluene in Waste Gases Using A Biological Trickling Filter." *Biodegradation 6*:109-118

Peters, D.A., G.T. Hickman, J.G. Stefanoff, and M.B.Garcia, Jr. 1993. "Laboratory Assessment of Biofiltration for Fuel-derived VOC Emissions Control," paper no. 93-WA-52B.06, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18.

Pettigrew, C.A., B.E. Haigler, and J.C. Spain. 1991. "Simultaneous Biodegradation of Chlorobenzene and Toluene by a *Pseudomonas* Strain." *Appl. Environ. Microbiol. 57*(1):157-162.

Phelps, T.J., K. Malachowsky, R.M. Schram, and D.C. White. 1991. "Aerobic Mineralization of Vinyl Chloride by a Bacterium of the Order *Actinomycetales*." *Appl. Environ. Microbiol. 57*(4):1252-1254.

Phelps, T.J., J.J. Neidzielski, R.M. Schram, S.E. Herbes, and D.C. White. 1990. "Biodegradation of trichloroethylene in continuous-recycle expanded-bed bioreactors." *Appl. Environ. Microbiol. 56*(6):1702-1709.

Radian/Envirogen. 1996. Final report for DOD contract F41624-95-C-8016, "2-Phase Extraction and Gas-phase Bioreactor Treatment of TCE in Soil and Groundwater." In press.

Rasche, M.E., M.R. Hyman, and D.J. Arp. 1991. "Factors Limiting Aliphatic Chlorocarbon Degradation by *Nitrosomonas europaea*: Cometabolic Inactivation of Ammonia Monooxygenase and Substrate Specificity." *Appl. Environ. Microbiol. 57*(10):2986-2994.

Reij, M.W., K.D. de Gooijer, J.A.M. de Bont, and S. Hartmans. 1995a. "Membrane bioreactor with a porous hydrophobic membrane as a gas-liquid contactor for waste gas treatment." *Biotechnol. Bioeng. 45*:107-115.

Reij. M.W., J. Kieboom, J.A.M. de Bont, and S. Hartmans. 1995b. "Continuous Degradation of Trichloroethylene by *Xanthobacter* sp. Strain Py2 During Growth on Propene." *Appl. Environ. Microbiol. 61*(8):2936-2942.

Revah, S., A. Hinojosa, E. Marroquin, and V. Morales. 1994. "Biotechnological Process for the Treatment of Hydrogen Sulfide and Carbon Disulfide in a Waste Gas." Presented at the First North American Conference on Emerging Clean Air Technologies and Business Opportunities. Toronto, Canada, September.

Revah, S., M. Acosta, W. Hugler, R. Trinidad, C. Avila, I. Estrada, and A. Hinojosa. 1995. "Air Biodesulfurization from Viscose Plants: Carbon Disulfide Elimination," *Proceedings of the 1995 Conference on Biofiltration*, University of Southern California, October 5-6:181-187.

Selifonov, S.A., M. Grifoll, R.W. Eaton, and P.J. Chapman. 1996. "Oxidation of Naphthenoaromatic and Methyl-Substituted Aromatic Compounds by Naphthalene 1,2-Dioxygenase." *Appl. Environ. Microbiol. 62*(2):507-514.

Semprini, L., G.D. Hopkins, P.V. Roberts, D. Grbić-Galić, and P.L. McCarty. 1991. "A Field Evaluation of In-Situ Biodegradation of Chlorinated Ethenes: Part 3, Studies of Competitive Inhibition." *Groundwater. 29*(2):239-250.

Semprini, L., P.V. Roberts, G.D. Hopkins, and P.L. McCarty. 1990. "A Field Evaluation of In-Situ Biodegradation of Chlorinated Ethenes: Part 2, Results of Biostimulation and Biotransformation Experiments." *Groundwater. 28*(5):715-727.

Shareefdeen, Z., B.C. Baltzis, Y-S. Oh, and R. Bartha. 1993. "Biofiltration of methanol vapor." *Biotechnol. Bioeng. 41*:512-524.

Shields, M.S., and M.J. Reagin. 1992. "Selection of a Pseudomonas cepacia Strain Constitutive for the Degradation of Trichloroethylene." *Appl. Environ. Microbiol. 58*(12):3977-3983.

Shields, M.S., M.J. Reagin, R.R. Gerger, C. Somerville, R. Schaubhut, R. Campbell, and J. Hu-Primmer. 1993. "Constitutive Degradation of Trichloroethylene by an Altered Bacterium in a Gas Phase Bioreactor." Presented at the 1993 Battelle In-situ and On-site Bioreclamation Conference in San Diego, CA.

Shields, M.S., S.O. Montgomery, P.J. Chapman, S.M. Cruskey, and P.H. Pritchard. 1989. "Novel Pathway of Toluene Catabolism in the Trichloroethylene-degrading Bacterium G4." *Appl. Environ. Microbiol. 55*:1624-1629.

Schraa, G., M.L. Boone, M.S.M Jetten, A.R.W. van Neerven, P.J.Colberg, and A.J.B. Zehnder. 1986. "Degradation of 1,4-dichlorobenzene by *Alcaligenes* sp. Strain A175." *Appl. Environ. Microbiol. 52*:1374-1381.

Smits, M.C.J., S.P.P. Ottengraf, and J.C. van den Heuvel. 1995 "Effect of the superficial liquid velocity on the nitrification of ammonia polluted air in a bio-trickling filter," *Proceedings of the 1995 Conference on Biofiltration*, University of Southern California, October 5-6:199-206.

Sorial, G.A., F.L. Smith, A Pandit, M.T. Suidan, P. Biswas, and R.C. Brenner, "Performance of Trickle Bed Biofilters Under High Toluene Loading," paper no. 95-TA9B.04, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23.

Sorial, G.A., F.L. Smith, M.T. Suidan, P. Biswas, and R.C. Brenner. 1994. "Evaluation of the Performance of Trickle Bed Biofilters - Impact of Periodic Removal of Accumulated Biomass," paper no. 94-RA115A.05, 87th Annual Air & Waste Management Association Meeting & Exhibition, Cincinnati, OH, June 19-24.

Spain, J.C., and S.F. Nishino. 1987. "Degradation of 1,4-Dichlorobenzene by a *Pseudomonas* sp." *Appl. Environ. Microbiol. 53*(5):1010-1019.

Speitel, G.E. Jr., and J.M. Leonard. 1992. "A Sequencing Biofilm Reactor for the Treatment of Chlorinated Solvents Using Methanotrophs." *Water Environ. Res. 64*(5):712.

Speitel, G.E., and D.S. McClay. 1993. "Biofilm reactors for treatment of gas streams containing chlorinated solvents." *ASCE J. Envir. Engrg. 119*(4):658-678.

Spiess, E., C. Sommer, and H. G`risch. 1995. "Degradation of 1,4-Dichlorobenzene by *Xanthobacter flavus* 14p1." *Appl. Environ. Microbiol. 61*(11):3884-3888.

Strand, S.E., M.D. Bjelland, and H.D. Stensel. 1990. "Kinetics of Chlorinated Hydrocarbon Degradation by Suspended Cultures of Methane-Oxidizing Bacteria." *Res. J. WPCF. 62*(2):124-129.

Strotmann, U.J. M. Pentegna, and D.B. Janssen. 1990. "Degradation of 2-chloroethanol by Wild Type and Mutants of *Pseudomonas putida* US2." *Arch. Microbiol. 154*:294-300.

Sutton, P.M., and P.N. Mishra. 1994. "Activated carbon based biological fluidized beds for contaminated water and wastewater treatment: a state-of-the-art review." *Wat. Sci. Tech.* 29(10-*11*:309-317.

Togna, A.P., and B.R. Folsom. 1992. "A Comparative Study of Biofilter and Biotrickling Filter Performance for Isopentane Removal," paper no. 92-116.04, 85th Annual Air & Waste Management Association Meeting & Exhibition, Kansas City, MO, June 21-26.

Togna, A.P., and M. Singh. 1994. "A Comparative Study of Biofilter and Biotrickling Filter Performance for Isopentane Removal," paper no. 94-RP115B.04, 87th Annual Air & Waste Management Association Meeting & Exhibition, Cincinnati, OH, June 19-24.

Togna, A.P., G.J. Skladany, J.M. Caratura, and S. Frisch. 1993. "Field-Pilot Results of Styrene Biodegradation Using Biofiltration: A Case Study," in *48th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Chelsea, MI:517-527.

Togna, A.P., G.J. Skladany, W.J. Fucich, W.J. Guarini, M. Singh, and T.S. Webster. 1995. "Removal of Isopentane and Isobutane Vapors from an Industrial Process Waste Stream Using a Field-pilot Biotrickling Filter," paper no. 95-TA9B.02, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23.

Togna, A.P., G.S. Skladany, and J.M. Caratura. 1994 "Treatment of BTEX and Petroleum Hydrocarbon Vapors Using a Field-Pilot Biofilter," in *49th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Chelsea, MI:437-448.

Torres-Cardona, M.D., S. Revah-Moiseev, A. Hinjosa-Martinez, F.J. Paez-Moreno, and V.M. Morales-Baca. 1993. "Biological process or the elimination of sulfur compounds present in gas mixtures." U.S. patent no. 5,236,677.

Tsien, H.C., G.A. Brusseau, R.S. Hanson, and L.P. Wackett. 1989. "Biodegradation of Trichloroethylene by *Methylosinus trichosporium* OB3b." *Appl. Environ. Microbiol.* *55*(12):3155-3161.

van den Wijngaard, A.J., K.W.H.J. Van Der Kamp, J. Van Der Ploeg, F. Pries, B. Kazemier, and D.B. Janssen. 1992. "Degradation of 1,2-Dichloroethane by *Ancylobacter aquaticus* and Other Facultative Methylotrophs." *Appl. Environ. Microbiol. 58*(3):976-983.

van den Wijngaard, A.J., R.D. Wind, and D.B. Janssen. 1993. "Kinetics of Bacterial Growth on Chlorinated Aliphatic Compounds." *Appl. Environ. Microbiol. 59*(7):2041-2048.

van der Ploeg, J., M.P. Smidt, A.S. Landa, and K.B. Janssen. 1994. "Identification of Chlorocataldehyde Dehydrogenase Involved in 1,2-Dichloroethane Degradation." *Appl. Environ. Microbiol. 60*(5)1599-1605.

van Groenestijn, J.W., and P.G.M. Hesselink. 1993. "Biotechniques for air pollution control." Biodegradation *4*:283-301.

van Lith, C., S.L. David, and R. Marsh. 1990. "Design criteria for biofilters." *Trans. IChemE. 68*(Part B):127-132.

van Lith, C.P.M., S.P.P. Ottengraf, and R.M.M. Diks. 1994. *Proceedings of the International Symposium on Biological Waste Gas Cleaning*, Heidelberg, Germany, March 9-11. VDI Berichte No. 1104:169-180.

van Lith, C.P.M., S.P.P. Ottengraf, B. Osinga, and R.M.M. Diks. 1993. "The Biological Purification of a Multi-Component Waste-Gas Discharged in Artificial Glass Production," paper no. 93-WA-52B.07, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18.

Vannelli, T., M. Logan, D.M. Arciero, and A.B. Hooper. 1990. "Degradation of Halogenated Aliphatic Compounds by the Ammonia-Oxidizing Bacterium *Nitrosomonas europaea*." *Appl. Environ. Microbiol. 56*(4):1169-1171.

VDI Richtlinie 3477. 1991. *Biological Waste Gas/Waste Air Purification, Biofilters.* Dusseldorf, VDI Handbuch Reinhaltung der Luft, Band 6.

VDI Richtlinie 3478. 1985. *Biological Waste Air Purification, Bioscrubbers.* Dusseldorf, VDI Handbuch Reinhaltung der Luft, Band 6.

Vembu, K., and C.S. Walker. 1995. "Biofiltration holds VOC odors at bay." *Environmental Protection.* February:27.

Vogel, T.M., C.S. Criddle, and P.L. McCarty. 1987. "Transformations of Halogenated Aliphatic Compounds." *Environ. Sci. Technol. 21*(8):722-736.

Wackett, L.P,. G.A. Brusseau, S.R. Householder, and R.S. Hanson. 1989. "Survey of Microbial Oxygenases: Trichloroethylene Degradation by Propane-Oxidizing Bacteria." *Appl. Environ. Microbiol. 55*(11):2960-2964.

Wackett, L.P., and D.T. Gibson. 1988. "Degradation of Trichloroethylene by Toluene Dioxygenase in Whole-Cell Studies with *Pseudomonas putida* F1." *Appl. Environ. Microbiol. 54*(7):1703-1708.

Wackett, L.P., and S.R. Householder. 1989. "Toxicity of Trichloroethylene to *Pseudomonas putida* F1 is Mediated by Toluene Dioxygenase." *Appl. Environ. Microbiol. 55*(10):2723-2725.

Wackett, L.P., M.J. Sadowsky, L.M. Newman, H.G. Hur, and S. Li. 1994. "Metabolism of Polyhalogenated Compounds by a Genetically Engineered Bacterium." *Nature. 368*:627-629.

Wark, K and C.F. Warner. 1981. Air Pollution: Its Origin and Control, 2nd Ed.; Harper and Row, Publishers, New York:405-423.

Weber, F.J., and S. Hartmans, "Toluene degradation in a trickle bed reactor - Prevention of clogging." *Proceedings of the International Symposium on Biological Waste Gas Cleaning*, Heidelberg, Germany, March 9-11, 1994. VDI Berichte No. 1104:161-168.

Webster, T.S., J.S. Devinny, E.M. Torres, S.S. Basrai, and V. Kogan, "Study of Biofiltration for Control of VOC and Toxics Emissions from Wastewater Treatment Plants: Phase II Bench-scale Experiments," paper no. 95-TA9B.06, 88th Annual Air & Waste Management Association Meeting & Exhibition, San Antonio, TX, June 18-23, 1995.

Wiedemeier, T., J.T. Wilson, D.H. Kampbell, R.N. Miller, and J.E. Hansen. 1995. "Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination in Groundwater." Prepared for the U.S. Air Force Center for Environmental Excellence (AFCEE), Technology Transfer Division, Brooks AFB, San Antonio, Texas.

Yang, Y., and D. Alibeckoff. 1995. "Biofiltration for Control of Carbon Disulfide and Hydrogen Sulfide Vapors," *Proceedings of the 1995 Conference on Biofiltration*, University of Southern California, October 5-6:165-179.

Yang, Y., and E.R. Allen. 1994a. "Biofiltration control of hydrogen sulfide 1. Design and operational parameters." *J. Air Waste Manage. Assoc. 44*(7):863-868.

Yang, Y., and E.R. Allen. 1994b. "Biofiltration control of hydrogen sulfide 1. Kinetics, biofilter performance, and maintenance." *J. Air Waste Manage. Assoc. 44*(11):1315-1321.

Yang, Y., A.P. Togna, and G. Skladany. 1994. "Treatment of CS_2 and H_2S Vapors by Biofiltration," in *49th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Chelsea, WI:449-456.

Yang, Y., A.P. Togna, and J.R. Blunk. 1993a. "Treatment of Carbon Disulfide by Biofiltration," Presented at the 1993 Superfund XIV Conference & Exhibition, Washington, D.C., Nov. 30-Dec. 2.

Yang, Y., J.M. Caratura, and D. Empfield. 1993b. "Use of Biofiltration to Control Hydrogen Sulfide Emissions," Presented at the 16th International Symposium on Gas, Oil, and Environmental Biotechnology, Colorado Springs, CO, Nov. 29 - Dec. 1.

Yavorsky, J.. 1993. "Biofiltration for Control of Gas Streams Containing Low Concentrations of Volatile Organic Compounds," paper no. 93-RP-139.02, 86th Annual Air & Waste Management Association Meeting & Exhibition, Denver, CO, June 13-18.

Ye, L., N.N. Khandan, and F. G. Edwards. 1994. "Biological Treatment of Airstreams Contaminated with Organic Vapors." *Wat. Sci. Tech. 30*(7):71-74.

Zahodiakin, P. 1990. "Puzzling out the new clean air act." *Chem. Enrg. 97*(12):24-27.

Zurlinden, R.A., J.C. Lucas, and C. Carmel, "Control of Gasoline-derived Volatile Organic Compounds by Biofiltration," paper no. 94-RA115A.06, 87th Annual Air & Waste Management Association Meeting & Exhibition, Cincinnati, OH, June 19-24, 1994.

Zylstra, G.J., L.P. Wackett, and D.T. Gibson. 1989. "Trichloroethylene Degradation by *Escherishia coli* containing the *Pseudomonas putida* F1 Toluene Dioxygenase Genes." *Appl. Environ. Microbiol. 55*(12):3162-3166.

APPENDIX A
LABORATORY WORK PLAN

This work plan describes the approach for the laboratory phase of the McClellan AFB Biological Treatment of SVE Off-gas demonstration. The work plan describes the system configuration and operation, method of introducing the vapor phase contaminants to the reactors, and analytical protocol, including sampling frequency and methods.

A.1 Development of a Contaminated Vapor Phase to Simulate the SVE Off-gas Contaminants

Six VOCs were selected from the contaminants detected in the SVE off-gas for the laboratory demonstration (Table A.1). They include PCE; TCE; 1,1,1-TCA; 1,2-DCB; toluene; and acetone. The gas-phase concentrations of the contaminants will similar to their average concentrations detected in the SVE off-gas stream and shown in Table A.1. The contaminated vapor stream will be created by dissolving the contaminants in water at predetermined concentrations, and stripping the water with air stream using a column packed with glass wool to create a contaminated gas stream.

TABLE A.1. CONTAMINANTS SELECTED FOR THE LABORATORY DEMONSTRATION

	Off-gas Conc.		
Compound	Max. (ppmv)	Avg. (ppmv)	H2O Concentration (mg/L)
PCE	97.4	63.0	0.403
TCE	111.3	74.4	1.07
1,1,1-TCA	241.4	152.9	4.15
1,2-DCB	120.6	35.5	2.82
Toluene	76.8	45.5	0.657
Acetone	92.0	63.8	180
Chlorinated Sum	571	326	8.44
Nonchlorinated Sum	169	110	180

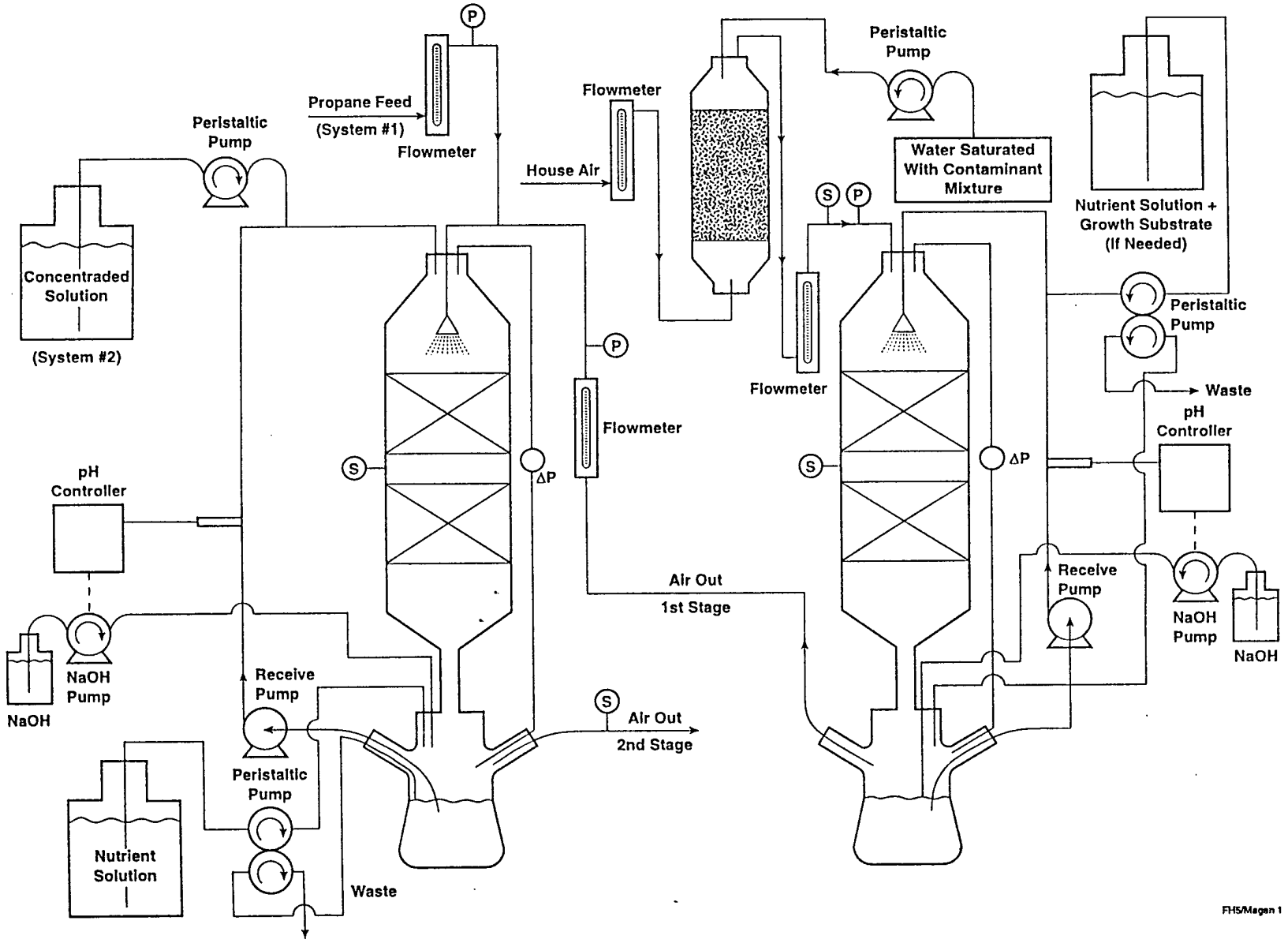

Figure A.1. Biotrickling Filter Reactor System DesignA.2 Reactor Configuration

The 2-stage laboratory-scale reactor systems are shown schematically in Figure A.1. Cosubstrates (propane or IPB) will be fed to the 2nd stage of each system. Propane will be added through a gas flow meter and will be monitored in the gas phase at the beginning and end of the second reactor stage to monitor the mass of propane consumed in the reactor. IPB will be added in the aqueous phase with the nutrients and will be metered into the reactor liquid phase using a peristaltic pump.

The reactors will consist of 4-ft tall, 1-ft diameter glass cylinders operated sequentially. The contaminant gas mixture will be fed to the reactors from an air-sparged contaminated aqueous solution. After the reactors are set up, they will be operated abiotically, without the introduction of biomass, to shake down the system for possible leaks, to determine whether there are abiotic losses in the system in the absence of microbial growth, and to assess the recovery of the contaminants in the system. The reactors will be operated without biomass for approximately 4 days.

After the abiotic control study, the reactors will be inoculated. If possible, propane-oxidizing bacteria and IPB-oxidizing bacteria will be obtained from Envirogen's laboratories, or other laboratories, to accelerate the growth and acclimation period during startup. The first stage reactors also will be inoculated, but specific biological enrichments will not necessarily be used.

Initially, the vapor flowrate through each system will be set to establish a vapor contact time of approximately 4 to 5 minutes in each stage. The system will be optimized in the laboratory for lower vapor contact times.

A.3 Reactor Sampling

Influent and effluent vapor concentrations will be monitored using an on-line gas chromatograph (GC). The online system will sample the reactors twice daily. Sampling ports will be located at (1) the influent to each system; (2) the effluent from the first stage of each system; and (3) the effluent from the second stage of each system. Each column will be equipped with at least one intermediate sample port to permit periodic grab samples. The online GC will sample the reactor influent and effluent streams twice daily to provide gas-phase VOC concentrations.

Three sets of liquid samples will be taken from each reactor stage throughout the duration of the laboratory demonstration. The liquid phase samples will be analyzed for aqueous-phase VOC and total organic carbon (TOC). The purpose of the liquid sampling will be to establish careful mass balances of the contaminants and cosubstrates in the reactors during the study. VOCs will be analyzed using EPA

Method 8260 and a GC/MS equipped with a purge-and-trap; TOC will be measured using EPA Method 415.1. Propane concentrations will be monitored in the vapor phase only, while IBP concentrations will be monitored in the aqueous phase. More frequent aqueous-phase IPB samples will be required to maintain target effluent IPB concentrations. Two times each week, liquid samples will be analyzed for chloride concentrations using an ion-selective probe.

Once a week, the vapor phase of the reactors will be sampled from the intermediate reactor sample ports in each stage. Grab samples will be taken with a syringe and injected directly into a GC/MS. The intermediate port samples will provide information on the degradation characteristics within the columns.

A.4 System Performance Under Stressed Conditions

Once steady state operating conditions are established, the system will be analyzed to evaluate its ability to handle shock loads. Shock loads will be imposed by rapidly (i.e., instantaneously) increasing the simulated-off-gas flow rates or off-gas VOC concentrations. The shock loads will be designed to mimic the full-scale system by increasing the reactor mass flow rates by the ratio of maximum to average off-gas contaminant concentrations, shown in Table A.1. During the stress tests, the online monitoring frequency of the reactors will be increased. Cosubstrate feed concentrations will be increased to maintain a constant cosubstrate/CAC feed ratio.

A.4 System Optimization

For the first 2 months of operation, no attempt will be made to optimize the system, although the cosubstrate/CAC ratios may be adjusted, if needed, to increase performance, and ethanol may be added to the 1st stage of each system to increase biofilm growth. In addition, a small amount of powdered activated carbon (PAC) may be added to the 2nd stage of each system to better regulate the supply of cosubstrate to the microorganisms. After the first 2 months, once steady-state has been achieved, the system will be optimized by increasing the vapor throughput until the predetermined DREs are no longer achieved; this will be the maximum vapor throughput. As the vapor flowrate is increased, the cosubstrate addition rate will be adjusted to maintain a constant cosubstrate/CAC ratio. Cosubstrate feeding also will be optimized by decreasing the cosubstrate/CAC ratio until DREs are no longer achieved; this will be the minimum possible cosubstrate/CAC ratio. The optimization period is expected to last for approximately 1.5 months. During that period, vapor and liquid sampling frequencies will be the same as described for the steady state operating period.

A.5 Quality Assurance

Envirogen's laboratory is certified by the New Jersey Department of Environmental Protection (NJDEP) for Method 8260 and 415.1 analyses. For these analyses, all laboratory QA/QC practices will be in accordance with NJDEP's Laboratory Certification Program. For the on-line vapor analyses, check standards with known concentrations will be analyzed weekly. The GC system will be calibrated if the measurements fall outside ±20% of the known check standard concentrations. In addition, influent and an effluent vapor samples will be collected at two time points using evacuated canisters for Method T0-14 analyses by an independent outside laboratory, selected by Battelle. Results will be compared to the on-line analyses. As a QA/QC check of the chloride measurements using the a chloride probe, the measurements from the probe will be compared with ion chromatography measurements performed by Envirogen's laboratory (Method 300), at four time points.